ENDOCRINOLOGY

THE BULLETIN OF
THE ASSOCIATION FOR THE STUDY
OF INTERNAL SECRETIONS

VOLUME 26 APRIL, 1940 NUMBER 4

QUANTITATIVE STUDIES ON THE HORMONES OF HUMAN PITUITARIES[1]

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THANKS TO THE COURTESY and cooperation of the Pathology Department of the Medical School of our university[2] we have been in a position to study the hormonal contents of over one hundred human hypophyses which were preserved during autopsies performed within 24 hours after death. There exists a considerable literature reporting hormonal tests made with a small number of glands which were implanted whole or cut in pieces. A few investigators also prepared and injected extracts. Both methods are apt to give incomplete and irregular results due to imperfect resorption, accidental fractionation and loss or augmentation of gonadotropic activity.

In our work on the comparative physiology of the pituitary of all vertebrate classes (1) the adoption of a standard technic which would permit comparison of pituitary substances of dissimilar types and collected at different times was an important prerequisite. After attempts with various extraction methods had proved unsatisfactory we resorted to the simple method of directly assaying the powders prepared from acetone-dried fresh glands. It is especially well suited for the testing of human pituitaries which, generally, are highly potent. The anterior lobes are cut in pieces and dropped into at least 5 times their volume of acetone. Later the acetone is changed, then poured off and the gland dried and pulverized. For the purpose of assaying, the desired amount of powder is taken up in distilled water and injected into the various assay animals. At all times the gland materials are stored in the ice box below a temperature of 5°C. This method provides not only a common and identical basis for all comparative purposes but also preserves the hypophyseal hormones quantitatively. Assays of the same powders repeated at intervals scattered, in some instances, over a period of 2 years give identical results. The acetone treatment has no augmenting effect. On this subject we shall soon report separately.

RESULTS

A. *Gonadotropic hormones.* The accompanying graphs (fig. 1, 2) contain the results of the assays of adult hypophyses in rat units. In some cases exhaustion of the ma-

[1] Aided by grants from the National Research Council, Committee for Research in Problems of Sex. Read before the 23rd annual meeting of the Association for the Study of Internal Secretions.

[2] We wish to express our gratitude to Drs. H. P. Smith, K. M. Brinkhous, E. D. Warner and other members of the staff of this department.

terial made a final determination impossible. This was especially true for hypophyses of children, which yield small amounts of powder and produce little gonadotropic activity. Assaying of 104 hypophyses resulted in 75 individual cases in determination of the exact amount of powder representing 1 R.U. Unfinished determinations are not included in our graphs, though the available tests conform in a general way with the picture offered by the completed cases. Establishing the rat unit requires at least 4 tests at various levels. Not counting the controls, a total of 375 tests on rats were made in this investigation. In addition the gonadotropic properties were studied also in various augmentation tests and by injections in finches.

In our experience ovarial weight is a very unsatisfactory index of gonadotropic activity. Enlargement of the uteri is certainly a more reliable and more convenient

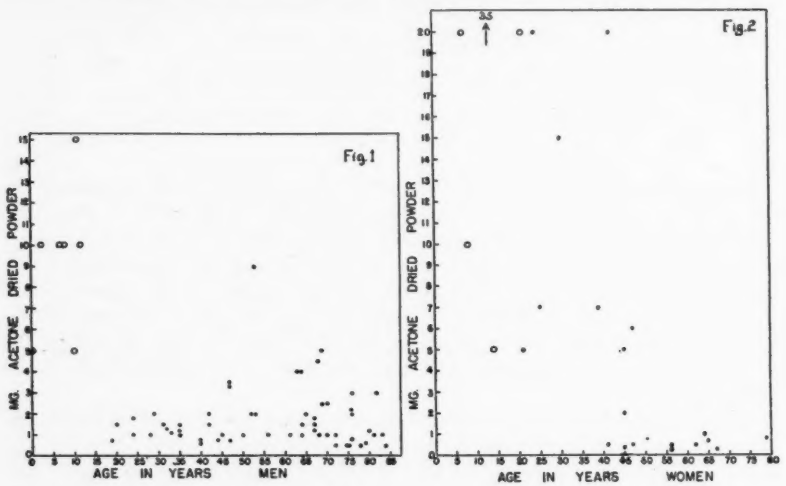

Fig. 1. Assays of gonadotropic potency of the anterior lobe of the hypophysis in 7 boys and 56 men. Black dots indicate the minimal amount of powder necessary to produce cornification of the vaginal epithelium in the immature female rat. Circles indicate amounts tested which failed to produce an effect. Fig. 2. Gonadotropic potency of female hypophyses. Only negative reactions were obtained from 3 girls while in a fourth one (age 13) the minimal R.U. was found at 35 mg. In 3 pregnant women, the R.U. was 20 mg. or more; in one woman, 3 months after delivery, it was 15 mg. Five non-pregnant women of the reproductive age (21 to 47) showed values from 5 to 7 mg. During the climacteric change a value of 2 mg. was found. A castrate (age 41) and 9 post-menopausal women (age 45 to 79) have highly potent hypophyses (1 R.U. per 0.25 to 1.0 mg.).

indicator; but we find the vaginal epithelium superior due to the qualitative changes which it exhibits as a consequence of ovarial stimulation (fig. 3). Only preparations which contain a very high amount of luteinizing hormone (LH) produce an increase in ovarian weight anteceding the attainment of stage 3 in the vaginal epithelium. In our laboratory we use the following routine technic. Immature female rats of a Wistar Institute colony which on the twenty-first day weigh between 35 and 50 gm. are selected. They are injected twice daily for 3 days, and killed 24 hours after the last administration. The ovaries are weighed and sectioned, together with sample pieces of uterus and vagina. Attainment of stage 3 in the vaginal epithelium marks the rat unit. This, in all probability, is a measure for the follicle stimulating fraction (FSH) only, although the possible augmenting effect of LH admixtures still needs further investigation. Unit weights always refer to total (not daily) amounts injected.

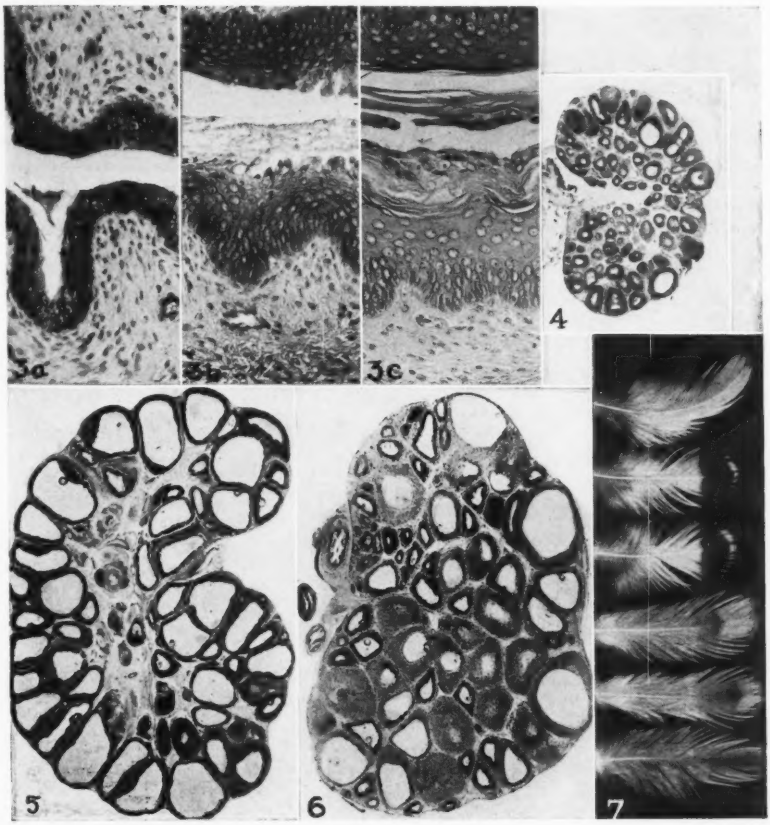

Fig. 3, a, b, c. SECTIONS THROUGH THE VAGINAL EPITHELIA OF 3 IMMATURE RATS INJECTED WITH 6, 8 AND 10 MG. OF ACETONE-DRIED POWDER OF THE HYPOPHYSIS OF A 56-YEAR OLD MAN. The lowest dose produces only a slight stimulation—below stage 1. The higher doses result in stages 2 and 3 (cornification). The R.U. of this case was finally fixed at 9 mg. ×155. Fig. 4. OVARY OF IMMATURE RAT WHICH HAD RECEIVED A TOTAL OF 5 MG. (ca. 1/6 R.U.) DRY POWDER OF ANTERIOR LOBE OF A 10-YEAR-OLD BOY. NO STIMULATION. Fig. 5. OVARY OF IMMATURE RAT STIMULATED WITH 5 MG. (10 R.U.) DRY POWDER OF ANTERIOR LOBE OF A 41-YEAR-OLD CASTRATED WOMAN. EXCESSIVE FOLLICULAR GROWTH. Fig. 6. OVARY OF IMMATURE RAT STIMULATED WITH 5 MG. (ABOUT 10 R.U.) DRY POWDER OF ANTERIOR LOBE OF A 65-YEAR-OLD WOMAN. In addition to follicular growth, formation of atypical, small corpora lutea were seen. The same hypophysis also produced a positive feather reaction in the weaver finch, indicating presence of some LH. Fig. 4, 5, 6 ×11 Fig. 7. COLOR REACTION IN FEATHERS OF THE WEAVER FINCH *Pyromelana afra* INDICATING THE PRESENCE OF LH. The first 3 are breast feathers, the last 3 are saddle feathers. Since the bird was a female, the permanent normal plumage is represented by the first (white) and the last (brown-buff) feathers. Administration of 20 mg. (ca. 50 R.U.) of hypophyseal powder of a 45-year-old menopausal woman, divided in 2 injections on consecutive days produced the positive reactions shown by the 4 feathers in the center. On the breast feathers there appear black bars; on the saddle feathers the melanin formation is inhibited and there appear lemon colored (carotinoid pigment) light bars. These simultaneous reactions represent a temporary reversal to the plumage type of the male in breeding season. ×1.2.

Before entering upon the discussion of the results, consideration must be given to the question of possible postmortem deterioration. For this purpose we refer to table 1 which contains data concerning hypophyses from old (above 45) and young (19 to 45) men, with distinctly high or low gonadotropic content. It shows no apparent

TABLE 1. MALE HYPOPHYSES WITH HIGH AND LOW GONADOTROPIN CONTENT

No.	Age, yr.	Month of death	Autopsy, hr. p.m.	Mg. ≙ gontr. R.U.	Mg. ≙ lactog. P.C.U.	Conditions
			Old men, low content			
39-64	53	3	5	9.0	0.5	Lobular pneumonia; respiratory failure
38-34	68	2	2	4.5	1.5	Neoplasm of lumbar spine and sacrum
38-35	64	2	1½	4.0	0.5	Renal adenocarcinoma, metast.; lob. pneumonia
38-90	47	4	4	3.5	1.0	Coronary occlusion
38-60	63	3	2½	3.5	1.5	Post-tubercular, bronchial pneumonia
38-61	70	3	14	3.5	1.2	Lob. pneumonia, senile atrophy of testes
38-132	47	5	9	3.0	0.8	Skull fract.; bronch. pneu.; hyperpl. prostate
38-22	69	1	11	2.5	1.0	Carcinoma of vocal cord, bronchial pneumonia
38-26	59	1	2	2.5	0.5	Neuro-syphilis, paresis; atrophy of testes
38-145	52	5	1	2.0	1.5	Necrotizing cellulitis, perineum, scrotum and l. back, from injury
38-103	53	4	2	2.0	1.5	Cirrhosis of liver, bronchial pneumonia
38-139	65	5	7	2.0	0.5	Subacute endocarditis, lobular pneumonia
37-241	76	9	6	2.0		Carcinoma of prostate, metast.; uremia
			Young men, low content			
38-175	29	6	2½	2.0	2.0	Rheumatic heart disease
38-58	35	3	2½	2.0	1.0	Malignant lymphoma (Hodgkin's); pulm. tuberc.
38-161	42	3	14	2.0	1.0	Ruptured mycotic aneurysm of thoracic aorta
37-147	20	5	3	1.5	2.0	Chorioepithelioma; r. testis removed 4 mo. before death, metastases to lung and brain
			Young men, high content			
38-131	24	5	2	1.0	0.8	Adenocarcinoma of rectum, lob. pneumonia, atrophy of testes
37-120	28	4	6½	1.0	1.5	Hydronephrosis; intracerebral lesion
37-322	32	12	22	1.0	2.0	Meningo vasc. syphilis, convolutional atrophy of brain
38-283	33	10	4½	1.0	1.0	Acute left ventricular failure
38-57	35	2	2	1.0	0.5	Medulo-blastoma of 4th ventricle; hydrocephalus
37-256	40	9	1	1.0	0.8	Meningitis, possibly epidemic
39-34	40	2	2	0.8	0.5	Attempted suicide, died bronch. pneumonia; testes normal
			Old men, high content			
37-337	61	12	24	0.5	0.8	Bronchiogenic carcinoma of r. lung; lob. pneu.
37-304	72	11	3	0.5	1.5	Hernia, intest. obstruction
38-169	75	6	6	0.5	0.5	Adenocarcinoma of prostate, lobular pneu.
39-63	75	3	3		0.5	Carcinoma of rectum, peritonitis; genitalia normal
37-336	84	12	17	0.5	0.5	Resection adenocarcinoma of prost.; pneumonia

gontr., gonadotropic hormone
lactog., lactogenic or mammotropic hormone
P.C.U., pigeon crop unit
p.m., postmortem
R.U., rat unit

correlation between potency of the hypophyses and time when they were preserved. This may be partly due to the fact that bodies which do not go immediately to the autopsy room are kept at low temperatures. Under these circumstances it is not surprising that equally favorable conditions of preservation are present in summer and winter months.

The first result of particular interest is the comparatively low amount of gonadotropins in the hypophyses of children (table 2). None of the hypophyses from

TABLE 2. ASSAY OF HYPOPHYSES OF CHILDREN, 0 TO 14 YEARS OF AGE

No. and sex	Age	Amount of powder	Reactions	
			Gontr.	Lactog.
	yr.	mg.		
37-280 ♂	stillborn	5	−	
37-226 ♂	2.5	10	−	
37-140 ♂	7	10	−	
		10	−	
39-30 ♀	7	20	−	
		1.0		+
		0.5		+
		0.25		+
38-96 ♂	8	10	−	
		5	−	
37-69 ♀	8	10	−	
		5	−	
38-79 ♂	10	5	−	
39-17 ♂	11	15	−	
		10	−	
		1		+
38-191 ♂	12	10	−	
		5	−	
		5		+
		0.5		+
		0.1		−
38-155 ♀	13	35	+	
		5	−	
38-253 ♂	14	5	−	
mixed	0–12	41	+	

newborn children and those up to 14 years of age produced a reaction when 5 mg. of dry powder was used. In fact the only two positive results indicate that about 35 mg. represents the characteristic dose for 1 R.U. Whether this value is constant throughout childhood remains uncertain. Due to the lack of material between the ages of 14 and 19 years no information is available concerning the changes during adolescence. The low content comes as a surprise since hypophyseal powders from immature calves and rats are equally or even more potent than those from the adults of their kind. It is probable that the prolonged juvenile period in man is in one way or another the cause of this peculiarity. It also seems probable that in all 3 species

the release of gonadotropin is low and that they differ merely in the amounts which are stored in the pituitaries.

Hypophyses of young men (age 19 to 45) are highly potent, the 16 cases of this group yielding from one-half to slightly over one full R.U. per mg. This is a very high potency in comparison with most animal hypophyses. Pituitaries of old men (45 to 84) give more fluctuating results. We cannot find any correlation between low and high gonadotropic values on the one side and the general physical condition or the cause of death on the other. Prostatic hypertrophy occurs in both extreme classes listed in table 1. It is, however, conceivable that low potency may be an expression of senile atrophy of the hypophysis while the general increase in potency after the age of 60, and especially the attainment of values above those of young men,

TABLE 3. HYPOPHYSES OF NON-PREGNANT WOMEN BETWEEN 20 AND 55 YEARS OF AGE

No.	Age, yr.	Month of death	Autopsy, hr. p.m.	Mg. ♀, gontr. R.U.	Mg. ♀, lactog. P.C.U.	Conditions
37-165	39	6	7.5	7.0		Bilateral mastoiditis, death after operation
37-332	25	12	2.5	7.0	0.5	Lupus erythematosus, hyperplasia of bone marrow
37-254	47	9	2	6.0		One ovary removed (1931); menses reg. to age 40, irreg. at 41, no later reports. Streptococcic meningitis, arthritis; confined last 16 years
37-72	45	3	2.5	5.0		Submucous fibroid; bronch. pneumonia; salpingectomy at 25, irreg. excessive menst., last one 20 days before death
37-318	21	11	3	5.0	1.0	Died of Hodgkin's disease general involvement of lymph nodes. Menses previously regular but none during last 11 months
38-160	45	6	2	2.0	2.0	Diabetes for 19 years, no treatment; died of gangrene; amputated r. leg. 4 children; last 4 to 5 yr. menorrhagia and metrorrhagia (prolonged bleeding, 2 to 3 wk. and 1 wk. interval); fibroid of uterus, several small cysts in ovaries
38-31	55	2	6	0.5		Adenomata of thyroid, small cyst in r. ovary; 7 children 12 to 32 yr. of age; no menstr. last 5 months
38-82	41	3	5.5	0.5	1.5	Cystadenocarcinoma of ovary, myxed. goiter, castrated 1931, still tumor at p.m., syphilis Wass. 1 to 2+, Kahn 4+
38-176	45	6	4	0.4	0.8	Hemorrhage at base of brain; arthritis; ovaries fibrotic; no menstr. last 3 years

may be due to atrophy of the testicular endocrine gland, resulting in a castrate condition of the hypophysis. It is of interest that even beyond the age of 80, half of the male hypophyses are within the normal range for young men.

The only hypophysis of a man with epithelioma mixtum (*case* 37–147, table 1) which we have assayed, was within the general range of men of his age. This was a typical case of so-called chorioepithelioma. The right testis had been removed 4 months before death, which was due to extensive metastases in the lungs and the brain. The rapid and fatal progress as well as the histological picture of the metastases suggest that this was one of the hormonally active cases; though no urine analysis had been made. The normalcy of this hypophysis therefore cannot support Hamburger's statement (2) that these hypophyses (similar to those of pregnant women) have been "found to contain no gonadotropic hormone." Further quantitative work in this field is still necessary. None of the 30 men with testicular tumors whose urines we have assayed during the past 3 years came to autopsy. It is of

interest that only 12 of these cases or 40% produced more than 10 R.U. of gonadotropic hormone per liter.

Relatively few hypophyses of women of the reproductive age were available (fig. 2). The first 5 of the non-pregnant cases listed in table 3 indicate that the female hypophyses are only about one-fourth as potent as those of men of the same age class. During pregnancy the contents in gonadotropic hormone drop further (table 4). It is of interest that the hypophysis of the fifth week of pregnancy, when the gonadotropic output is at its maximal height, shows the same potency (20 mg. = 1 R.U.) as another one of a women who died during delivery, that is at the time when the pregnancy gonadotropin is lowest. The fact that the gland from the end of the third month was least potent (20 mg. give an entirely negative result) suggests that the hypophysis is progressively inactivated during about the first half of pregnancy and that recovery begins during the second half. Recovery seems to be slow, judging from the relatively low potency of the gland from a woman who died 3 months after delivery; positive tests were obtained only at the rate of 15 mg. per R.U. Obviously there exists no straight parallel with the concentration of chorionic gonadotropin nor with that of the estrogens of pregnancy.

Three nonpregnant women below the age of 50 have surprisingly potent hypophyses (table 3). Of these a 41-year-old woman had both ovaries removed 7 years before death occurred. The ovaries of the other 2 cases were fibrotic; in one case it was known that menstruation had stopped 3 years before death. These two are obviously instances of early but essentially normal menopause. The castrate is of particular interest in view of the picture presented by the hypophyses of all postmenopause women (fig. 2). All cases, 9, of this latter type are of high potency, 1 R.U. being contained in 1 mg. or less. It appears that on the average the female hypophysis after castration or after menopause contains about 10 times as much gonadotropin as in the normal, nonpregnant condition during the reproductive phase. The average weights of dry powder representing 1 R.U. are 0.5 and 5 mg. respectively.

The changes occurring in the climacteric are illustrated by 2 women listed in table 3. One died at the age of 45 after she had suffered from 4 to 5 years of menorrhagia (*case 38-160*). The potency of the hypophysis (2 mg. = 1 R.U.) from this patient was intermediate between that of young and old women. The second patient was a 55-year-old woman with a small ovarian cyst and an irregular menstrual history during her last years (*case 38-31*). She had a last (normal?) menstruation 5 months before her death. The hypophysis was entirely of the castrate type (0.5 mg. = 1 R.U.).

Figures 4 and 5 show side by side the entirely negative response of a rat ovary to injection of 5 mg. powder of a child's hypophysis and the strong reaction following the administration of the same amount of hypophysis from the castrate woman, 41 years old. The excessive formation of large follicular cysts is the characteristic response to the injection of moderately large amounts (about 10 R.U.) of human pituitary. In the present case the injection of only 1 mg. (2 R.U.) induced abundant ovulation. The higher amount obviously caused a rapid development of the follicles past the ovulation stage. The absence of typical corpora lutea in most of these ovaries seemed to suggest that the human hypophysis contains relatively little luteinizing hormone (fig. 6). This indication was followed up by tests specially developed to qualitatively separate FSH and LH action. A more complete report on this subject will be published elsewhere, it suffices to mention here only 2 tests, one to prove the high content of FSH, the other the near absence of LH.

In finches the so-called follicle stimulating hormone accelerates spermatogenesis and ovogenesis but does not stimulate the endocrine glands within the testes and

ovaries. Consequently FSH treatment is followed by the enlargement of the gonads but not of the gonoducts. On the other hand, substances very rich in LH, such as hypophyseal powders of the turkey or the sheep cause a very rapid development of the gonoducts and a much slower one of the ovaries and testes. As shown in figure 8, human hypophysis produces nearly identical reactions as fractionated and purified follicle stimulating hormones.[3] On the other hand the luteinizing hormone causes directly and without the cooperation of either FSH or sex hormones a characteristic color reaction on regenerating feathers of weaver finches (3, 4). Of 14 vertebrate species so far tested, the human hypophysis is the only one which does not produce this reaction if administered in the amount of 1 R.U. So far 2 hypophyses were found which gave a characteristic reaction with the equivalent weight of 40 to 50 R.U. (fig. 7) and 3 more which gave indefinite reactions only. From most human hypophyses

TABLE 4. HYPOPHYSES OF PREGNANT WOMEN

Month of pregnancy	No.	Age, yr.	Month of death	Autopsy, hr. p.m.	Mg. ♀, gontr. R.U.	Mg. ♀, lactog. P.C.U.	Conditions
1	37-235	42	8	8	20	0.5	Died of glioblastoma of left temp. lobe, pregnant 4 to 5 weeks
3	37-242	21	9	5	more than 20		Miscarriage at 3 mo. (50 mm.), died 8 hours later
10	37-274	24	10	7	20	1.0	Latent syphilis, died during delivery after normal pregnancy
3 mo. after del.	37-279	30	10	12	15	1.0	Postpartum infection of uterus, hysterectomy, died of bacterial endocarditis

no effects could be obtained, even though up to 100 R.U. was injected. This third test reaffirms the conclusion that human hypophyses contain but traces of luteinizing hormones, in some cases probably none at all. Whether possibly they produce some substance similar to the chorionic hormone, known best from the pregnancy urine, is not decided by our tests, since the pregnancy hormone affects neither the development of the genital organs of sparrows nor the pigmentation of the feathers of weaver finches.

B. Thyrotropic hormone. We had hoped that on the basis of Miller's work (5) it would be possible to develop a finch unit for the assay of thyrotropic substances. The thyroids of these birds respond very strikingly and have the further advantage of remaining responsive for months or even permanently. However, on closer examination it proved impossible to eliminate a certain variability in the controls, which is very disturbing if one attempts to fix the dosage of a minimal unit. We have assayed a number of human hypophyses on sparrows and a total of 38 on canaries. As little as 1 mg. of powder almost invariably gave stimulated thyroids. At this level there is no difference between the age groups, including children, nor between pregnant and nonpregnant women. Quite obviously 1 mg. was still considerably above the minimal amount for most cases. In lowering the dose further the range of the just mentioned variability of control thyroids is encountered. While some individual differences exist between the tested glands we received the impression that they were not correlated with age or sex.

C. Lactogenic hormone. The content in lactogenic hormone was tested by the technic of local injections (blister method) above a pigeon crop gland. Some of the re-

[3] We are indebted to Dr. H. M. Evans for the FSH preparation used in this experiment.

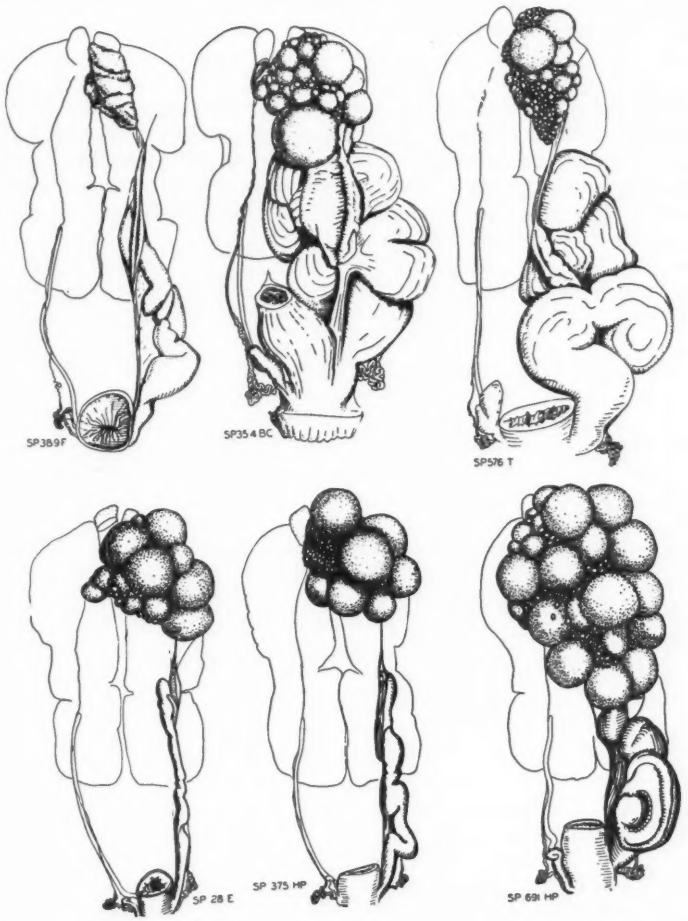

Fig. 8. UROGENITAL ORGANS (OVARY, OVIDUCTS, VASA DEFERENTIA) OF FEMALE SPARROWS. *Sp. 38.9F*, CONTROL FEMALE CAUGHT AND KILLED IN SEPTEMBER; QUIESCENT SEASON; SMALL OVARY, SMALL GONODUCTS. *Sp. 35.4 BC*, CONTROL FEMALE CAUGHT AND PRESERVED IN APRIL; OVARY AND GONODUCTS ENLARGING, AS TYPICAL FOR BEGINNING BREEDING SEASON. *Sp. 576T*, FEMALE OF THE QUIESCENT SEASON STIMULATED BY THE INJECTION OF DRY POWDERED TURKEY HYPOPHYSIS; DUE TO THE PREVALENCE OF LH, THE GONODUCTS ARE IN PROPORTION MORE STIMULATED THAN THE OVARIAN FOLLICLES. *Sp. 28E*, QUIESCENT FEMALE STIMULATED WITH A FSH PREPARATION BY H. M. EVANS; ONLY THE OVARIAN FOLLICLES ARE STIMULATED. *Sp. 375 HP* AND *691 HP*, 2 QUIESCENT FEMALES STIMULATED WITH UNFRACTIONATED POWDER OF HUMAN (OLD MEN'S) HYPOPHYSES (21.5 mg., ca. 43 R.U., 10 DAYS) RESP. (24 mg., ca. 50 R.U., 12 DAYS). MAINLY FOLLICLE STIMULATION. ALL CAMERA LUCIDA DRAWINGS, ×2.4.

sults are given in tables 1 to 4. There exists no obvious correlation between age, sex or pregnancy and the lactogenic potency. Children's hypophyses, like those of all adult classes, showed values between 0.5 and 2 mg.

D. Chromatophorotropic hormone. The content in chromatophorotropic hormone was found constant, changing neither with age nor sex. No assays were made with hypophyses from pregnant women. In all tests a dose of 0.1 mg. gave a positive

chromatophore-dilating reaction in *Rana clamitans* larvae. Larger amounts give quicker and more extensive effects.

DISCUSSION

The first tests for gonadotropic hormones in human hypophyses were probably made by Zondek and Aschheim (6). Later several investigators (7, 8, 9) obtained significant results in the direction of quantitative measurement and comparison of glands of different types. Philipp (10) found hypophyses of 3 pregnant women less potent than those of a castrated and of 2 post-menopausal women. Wirz (11) noticed indi-

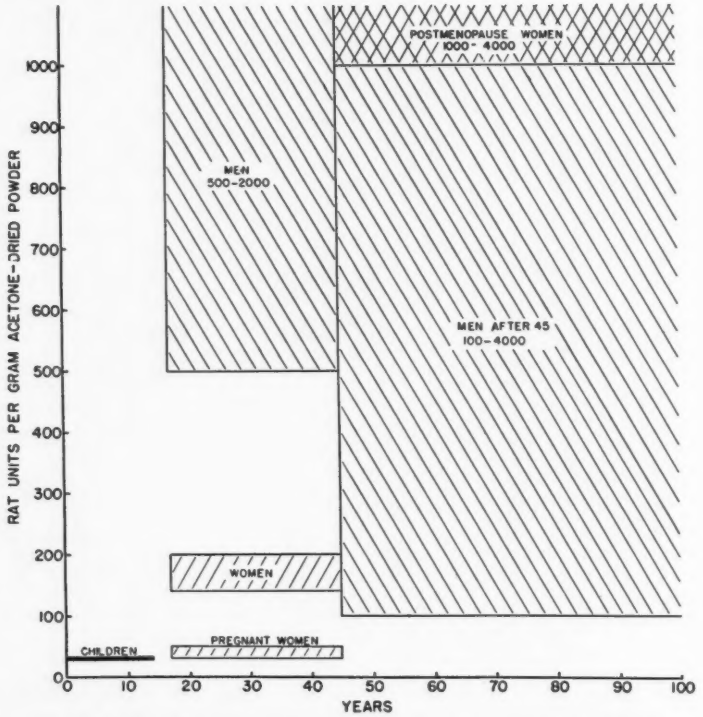

Fig. 9. CONTENT IN GONADOTROPIC PRINCIPLE PER GM. OF ACETONE-DRIED POWDER.

cations of recovery of higher potency during 5 weeks following delivery. Loeb and his coworkers (7, 8) were first to establish the fact of a relatively low potency in children. All these investigators had used mainly the method of implantation of large pieces of fresh or preserved hypophyses. Correspondingly the results are only in a gross way quantitative and conclusions were often indefinite or in some instances decidedly wrong. Previous attempts to discriminate between follicle stimulating and luteinizing actions have not produced any real information, mainly due to the exclusive use of the ovaries of normal immature rodents as test material.

The gonadotropic content of the human hypophysis, due to the extreme preponderance of the follicle stimulating principle differs in character very greatly from

that of either cattle, sheep or pigs. Even horse hypophysis and pregnant mare serum are relatively richer in luteinizing principle though no doubt they are more suited for replacement therapy in human patients than crude, non-fractionated extracts of sheep hypophyses. Prephysin, Chappel, an extract from horse pituitary seems to come very close to the human composite (however, the authors have no knowledge concerning the contents of other hypophyseal hormones in this preparation).

A picture of the varying amounts of gonadotropic hormone, as expressed in R.U. per gm. acetone-dried powder is given in figure 9. In castrates and their physiological equivalents, post-menopausal women and many old men, up to 4000 R.U. may be present, but in children only about 30 R.U. The fresh weight of the anterior lobe of an old man's hypophysis, carefully cleaned of all envelopes and adhering tissues, is approximately 350 mg., yielding close to 70 mg. powder which may contain as much as 280 R.U. of gonadotropin. On the other hand a child's hypophysis scarcely ever contains more than one or at the most two R.U.

The change from low to high productivity, during adolescence, is, as far as we know, independent of other hormonal conditions. The hypophysis seems to play the leading rôle in bringing about maturity of the endocrine sex organs.

Quite to the contrary, the differences in potency encountered in the hypophyses during the reproductive phase are unmistakably due to the various amounts of sex hormones present. It is well known that the amount of circulating gynogenic substances is lowest in castrates and progressively higher in young men, young women and pregnant women. It appears that the concentration in female sex hormones alone is sufficient to explain all variations; though it is not excluded that male sex hormones similarly, but to a smaller degree, may repress the gonadotropic center in the hypophysis. Partial inhibition of endocrine functions by estrogens, even though of long duration, does not impair hormone production in a permanent way, for castrated and post-menopausal women reach as high or higher levels as any of the male group. Rather the total amount becomes increased since the adult female hypophysis is considerably larger than the male (12).

The low content found in the hypophyses of many old men may be due to a primary, not hormonally induced low activity of these glands.

Gonadotropic potency closely parallels the relative frequency of basophilic cells in a hypophysis. According to data of Rasmussen (12) and Severinghaus (13) typical basophils are increasing in frequency in the following order: children, pregnant women, young women, men, old women and castrates. The statement of Rasmussen that the old age increase of basophils is limited to women is of great interest and conforms well with the physiological assays presented in this paper. It would be desirable, however, to study individual old men with physiological as well as cytological methods. Contrary to certain views expressed in the vast literature on this subject, we get the impression that the content of follicle stimulating hormone in the hypophysis runs parallel with its output and its concentration in blood and urine. The gonadotropic hormone of pregnancy, however, is not found in demonstrable quantity in the hypophyses of pregnant women, an observation distinctly in favor of the assumption of its extra-hypophyseal origin.

The absence of significant differences in thyrotropic and chromatophorotropic contents in all age groups is not surprising in view of the importance of these hormones for the metabolism of all stages. Rather unexpected, however, is the finding of an equally constant distribution of the lactogenic hormone. It remains unexplained, unless we adopt the view that this hormone is also of some general and presumably metabolic importance.

SUMMARY

Pulverized acetone-dried human hypophyses were assayed in various tests with the following results. No distinct variations according to age and sex were found with respect to the thyrotropic, the lactogenic and the chromatophorotropic hormones. The gonadotropic hormone is present only in small quantities in children; amounts of powder below 30 mg. produced no reactions in the immature rat. In the reproductive age the hypophyses of men are about 4 times more potent than those of women. Old women (over 50) and castrates have hypophyses of highest potency. The hypophyses of old men give varied values, some of them also assuming the castrate type. During the first period of pregnancy the gland rapidly loses potency; regaining it slowly toward the end, and more rapidly after delivery. The human gonadotropic complex is unusually rich in FSH while LH is present only in traces.

REFERENCES

1. Witschi, E.: *Symp. Quant. Biol., Cold Spring Harbor* 5: 180. 1937.
2. Hamburger, Ch.: *Actualites Sci. et Industr.* 605: V-345. 1938.
3. Witschi, E.: *Proc. Soc. Exper. Biol. & Med.* 35: 484. 1936.
4. Witschi, E.: *Internat. Congr. Physiol. Zürich.* 2: 315. 1938.
5. Miller, D.: *J. Exper. Zool.* 80: 259. 1939.
6. Zondek, B., and S. Aschheim: *Arch. f. Gynäk.* 130: 21. 1927.
7. Kunkel, P., and L. Loeb: *Proc. Soc. Exper. Biol. & Med.* 32: 1413. 1935.
8. Saxton, J., and L. Loeb: *Anat. Rec.* 69: 261. 1937.
9. Zondek, B.: Berlin, 1931.
10. Philipp, E.: *Zentrlb. f. Gynäk.* 54: 450. 1930.
11. Wirz, P.: *Z. f. Gebhülfe u. Gynäk.* 104: 293. 1933.
12. Rasmussen, A. T.: *Proc. Assoc. Res. Nerv. & Mental Disease* 17: 118. 1936.
13. Severinghaus, A. E.: Sex and Internal Secretions. Williams & Wilkens, Baltimore. 1939.

ANTIBODY RESPONSE IN RABBITS TO EXTRACTS OF HUMAN PREGNANCY URINE AND TO EXTRACTS OF NORMAL FEMALE URINE[1]

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ASCHHEIM AND ZONDEK (1) in 1928 described the hormones in the urine of pregnant women. Since that time numerous investigations have been made on the effect produced by the injection of these hormones into animals. From the reversal in effect which occurs after continued administration of the urinary extracts, (2–10), it is apparent that an antagonistic factor is elicited in the serum of the experimental animals. There is divergence of opinion as to the nature of this inhibitory substance. The following are the chief possibilities that have been suggested: (*a*) an antihormone in the nature of a true antibody (3, 4, 5, 11–13); (*b*) a non-specific urinary antibody (6, 10); (*c*) both antihormone and non-specific urine antibody present (6, 14, 15); (*d*) a factor closely allied to immune bodies (16).

An unusual opportunity to investigate the serologic reactions elicited by injections with pituitary-like hormone in the urine of pregnant women was made possible by the interest and cooperation of Doctor Oliver Kamm, Scientific Director of Parke, Davis & Company. The gonadotropic hormone used in the experiments was antuitrin-S (Parke, Davis & Co.), a highly purified extract of human pregnancy urine; 50 to 100 cc. of urine, depending upon potency, are necessary to produce 1 cc. of the product; one cc. is standardized to contain 100 or more rat units per cc. Doctor Kamm furnished the following products: (*a*) a heat-inactivated product identical to the antuitrin-S preparation except that it had been subjected to heat until it was substantially inactive by the rat-luteinizing test; (*b*) a normal female urine extract, each cc. representing 75 cc. of urine, produced under the exact conditions under which antuitrin-S was prepared and considered practically inert since each cc. had only a potency of 0.15 R.U.

When the normal female urine extract was received, a study of the serum response of patients treated with gonadotropic hormone (17) was nearing completion, and only two serums were still available. Complement-fixation tests on these two serums were made, using the normal urinary extract as antigen, and resulted in 4-plus positive reactions. This result was similar to that obtained previously when the pregnancy urine extract, used as antigen, had suggested a specific antigen-antibody response.

Because of the difficulty in obtaining serum from patients treated with gonadotropic hormone and because experiments on animals could be more easily controlled, it was decided to study the effects on the blood of rabbits injected with antuitrin-S, heat-treated antuitrin-S and the extract of normal female urine prepared by Dr. Kamm.

This study is limited in its scope to the determination as to whether the antigonadotropic factor is a specific antihormone in the immunologic sense, or whether it is a non-specific urinary antibody.

[1] Aided by the A. B. Kuppenheimer Fund.

Seventeen rabbits were used in the experiments; these were divided into 4 groups—6 rabbits were used in the first, 3 in the second, 3 in the third, and 5 in the fourth. All rabbits were half-grown, young and healthy, weighing about 4 lb. In the first group 2 rabbits were injected with the extract of normal urine, 2 with antuitrin-S and 2 with the heat-treated antuitrin-S (a male and a female rabbit each time). Each was injected intravenously daily for 5 days. The initial dose was 0.5 cc., and subsequent doses were increased to 1 cc. or even, in a few instances, to 2 cc. The rabbits were bled from the heart on two separate occasions, 4 days and again 10 days after the last injection, and the serums tested in vitro for immune bodies. After a rest period of 10 days to 3 weeks, a second series of 5 injections of the respective antigens were administered and tests on the serums were performed.

The amounts of antigen used in the first and second series of injections were usually 3 to 4 cc. and 5 cc. respectively, a total of 7 to 10 cc. of antigen for each rabbit. The urinary extracts had little toxicity for rabbits as indicated by blood counts and mortality. To judge whether or not the state of health of the rabbits had any influence on the results, initial blood counts were made before injection of the antigens and at frequent intervals thereafter. Occasionally a mild secondary anemia developed, which might have been due to the injections or to other factors such as bleeding, food and environment. A characteristic blood count over a period of immunization (*rabbit 1*) was as follows.

R.B.C.	W.B.C.	Hgb.
5,560,000	7,650	90
5,560,000	6,725	85
5,210,000	8,900	85
4,160,000	5,100	80

Only 3 rabbits died during the course of the experiment; and of these, two succumbed to intracurrent infections while one death occurred after a massive withdrawal of blood from the heart.

The in vitro immunologic tests used were complement-fixation and precipitin tests, and the in vivo one, a skin test. Complement-fixation tests were performed according to the original Wassermann reaction in 0.1 the amount. The two quantitative tests were made upon each serum; in one the antigen was the variable factor, and in the other the serum. Each serum was tested against the 3 urinary antigens (antuitrin-S, heat-treated antuitrin-S, and the extract of normal female urine), against a Kolmer Wassermann antigen as a control, and without antigen of any type. Control serums were included with all tests; the negative controls were serums from the rabbits before injection, and the positive controls at first a strongly positive patient's serum and later (as soon as it was available) a strongly positive rabbit serum. Precipitins were tested by the contact method. Increasing dilutions of each antigen made with physiologic salt solution and a constant amount of clear, undiluted serum was used. The tests were read immediately and then after 1 hr. incubation at room temperature. The controls used were the same as for the complement-fixation tests. When antibodies were demonstrated by the in vitro serologic tests, intradermal injections were made with the homologous and heterologous extracts, and with normal sterile saline as a negative control.

The results of the serologic tests are recorded in table 1. The highest titer of serum obtained at any time during the period of observation is the one recorded in the table. This usually was the sample of blood obtained 10 days after the second series of injections. Examination of the table indicates that there is no specificity in the reactions. An immune serum reacted as strongly positive with the two heterologous antigens as with the homologous one. The complement-fixation test was more

sensitive than the precipitin test, a fact also noted by Bachman (6). The precipitin reactions were negative or very weakly positive. Insofar as any conclusion can be drawn, they were non-specific. In most instances the skin reactions were essentially negative, but there was an occasional slight reddening and a little edema surrounding the point of inoculation. *Rabbit 2*, injected with the normal urinary extract, had a definite 3-plus positive reaction with the homologous antigen, a one-plus positive with antuitrin-S, and a trace only with heat-treated antuitrin-S. In contrast to this fairly specific skin reaction, *rabbit 8*, treated with antuitrin-S, had a 3-plus positive reaction with heat-treated antuitrin-S and negative with the homologous antigen and

TABLE 1. SEROLOGIC REACTIONS OF SERA FROM RABBITS INJECTED WITH VARIOUS URINARY EXTRACTS

Rabbit and sex	Amount of antigen injected	Antigens: Normal urinary extract C.F.T.	Normal urinary extract Precipitin	Antuitrin-S extract C.F.T.	Antuitrin-S extract Precipitin	Heat treated antuitrin-S extract C.F.T.	Heat treated antuitrin-S extract Precipitin
	Normal urinary extract						
1 ♀	9.00 cc.	‡‡‡‡	⊗	++	o	++++	o
2 ♂	8.50 cc.	‡‡‡‡	⊗	++++	o	+++	o
3 ♀	8.00 cc.	‡	⊗	++	o	o	o
4 ♂	5.25 cc.	⊗	⊗	o	o	o	o
5 ♀	3.00 cc.	‡	‡	+	o	o	o
6 ♂	7.00 cc.	‡‡‡‡	‡‡	++++	++	++	++
	Antuitrin-S extract						
7 ♀	7.25 cc.	++	o	‡‡	⊗	+++	o
8 ♂	7.75 cc.	++	o	‡‡	‡	++++	o
9 ♀	4.50 cc.	o	o	‡‡	⊗	o	o
10 ♂	2.50 cc.	o	o	‡	⊗	++++	o
11 ♀	9.00 cc.	+++	++	‡‡‡‡	‡‡	+++	++
12 ♂	8.00 cc.	+	+++	‡‡	‡‡	++	++
	Heat treated antuitrin-S						
13 ♀	4.00 cc.	++++	o	+++	o	‡‡‡	⊗
14 ♂	9.00 cc.	+++	o	++	o	‡‡	⊗
15 ♀	10.00 cc.	o	o	++	o	‡‡	⊗
16 ♂	6.00 cc.	++++	o	+	o	‡‡‡‡	⊗
17 ♀	3.25 cc.	o	o	+	o	‡‡‡	⊗

The results obtained with homologous antibody and antigen are indicated by ‡ and ⊗

the normal urinary antigen. This apparent non-specificity of the skin reactions corresponds to those obtained by other workers who concluded that skin tests with gonadotropic antigens were most uncertain. There was no difference in any of the immune reactions of male and female rabbits to the urinary extracts.

SUMMARY

The results of this study indicate that the complement-fixing antibodies and the precipitins irregularly detectable in the serums of animals which have been injected with extracts of urine from pregnant human females are antibodies to substances present in the urine and not specific antihormones in the serologic sense, because similar reactions are produced by urinary extracts that are practically free from hormone.

REFERENCES

1. Aschheim, S., and B. Zondek: *Klin. Wchnschr.* 7: 8. 1928.
2. Zondek, B.: Hormone d Ovariums u.d. Hypophysenvorderlappens, Berlin and Vienna, Julius Springer, 2nd. edition, 1935.
3. Collip, J. B., H. Selye and D. L. Thomson: *Proc. Soc. Exper. Biol. & Med.* 31: 487. 1934.
4. Collip, J. B., H. Selye, D. L. Thomson and C. Bachman: *Proc. Soc. Exper. Biol. & Med.* 31: 1113. 1934.
5. Collip, J. B., C. Bachman and H. Seyle, *Proc. Soc. Exper. Biol. & Med.* 32: 544. 1935.
6. Bachman, C.: *Proc. Soc. Exper. Biol. & Med.* 32: 851. 1935.
7. Fluhmann, C. F.: *Proc. Soc. Exper. Biol. & Med.* 32: 1595. 1935.
8. McPhail, M. K.: *J. Physiol.* 80: 105. 1933.
9. Smith, P. E.: *J.A.M.A.* 104: 548. 1935.
10. Sulman, F.: *J. Exper. Med.* 65: 1. 1937.
11. Ehrlich, H.: *Wien. klin. Wchnschr.* 47: 1323. 1934.
12. Gordon, A. S., W. Kleinberg and H. A. Charipper: *Science* 86: 62. 1937.
13. Bischoff, H. W., and W. R. Lyons: *Endocrinology* 25: 17. 1939.
14. Gustus, E. L., R. K. Meyer and J. H. Dingle: *Proc. Soc. Exper. Biol. & Med.* 33: 257. 1935–36.
15. Gegerson, H. J., A. R. Clark and R. Kurzrok: *Proc. Soc. Exper. Biol. & Med.* 35: 193. 1936.
16. Zondek, B., and F. Sulman: *Proc. Soc. Exper. Biol. & Med.* 37: 343, 1937.
17. Saphir, W., K. M. Howell and R. H. Kunstadter: *Endocrinology* 24: 182. 1939.

THE ACTION OF DIETHYLSTILBESTROL IN GYNECOLOGICAL DYSFUNCTIONS

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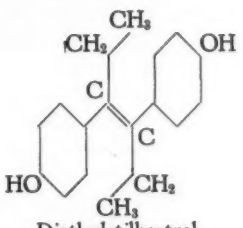
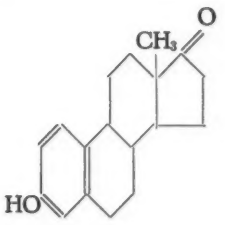
DODDS ET AL. (1, 2) described the estrogenic activity of certain derivatives of diphenylethane, and in particular it was shown that the introduction of an ethylene linkage between the α and β carbon atoms greatly increased the potency of the compounds. A number of stilbene derivatives were found to possess marked estrogenic activity. The most potent substance is 4, 4′-dihydroxy-α, β-diethylstilbene. It has the following activity as determined by the production of a vaginal estrous response.

	Dose in mg.	% Positive animals
In sesame oil	0.0005	100
	0.004	100
	0.0035	60
In water	0.0005	100
	0.0003	80
In oil, orally	0.001	80

It will be noted from this that the new compound is about three times as active as estrone (I.U. = .0001 mg.). In view of the fact that 4, 4′ dihydroxystilbene is the mother substance of a series of estrogenic agents, Dodds suggested that it be called "stilboestrol." In the following clinical experiments we used a derivative, diethylstilbestrol. The structural resemblance of this compound to estrone can be seen in the following formulae.

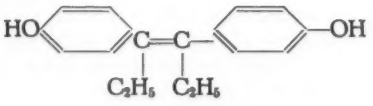


Diethylstilbestrol



Estrone

or



Physiological Action

Dodds, Lawson and Noble (3) studied the effect of diethylstilbestrol on the uterus of the ovariectomized virgin white rat. Definite growth of the uterus occurred

[1] E. R. Squibb & Sons Fellow in Endocrine Research.

with a dosage of 1γ: 50γ produced marked stimulation, while with 200γ the uteri were thin-walled and ballooned out with fluid. They also report diethylstilbestrol to be 2½ times as potent as estrone. On the contrary if growth of the mammary gland of a guinea pig be taken as an index of activity then stilbestrol is only ⅕ as active as estrone. Both estrogens had an equal effect on nipple growth.

Noble (4) studied the effect of subcutaneous insertion of diethylstilbestrol crystals on the activity of the anterior pituitary gland. Growth of the animals was inhibited for a short time. The ovaries remained in an infantile state. He also noted that the injection into rats of large doses of this substance (in oil solutions) is followed by atrophy of the testes, prostate and seminal vesicles in the male, ovarian atrophy in the female, and increase in weight of adrenals and pituitary of both sexes. The changes produced in water balance do not indicate that any alteration in the secretion of the diuretic principle of the anterior pituitary has occurred.

Parkes, Dodds and Noble (5) found that the oral administration of diethylstilbestrol suppressed the progestational action of progesterone (rabbit) and prevented the establishment of pregnancy (rabbit and rat).

Folley and Watson (6) found that diethylstilbestrol can partially inhibit the enlargement of the pigeon crop glands due to the administration of prolactin. It also inhibited lactation in rats, in a manner similar to the natural estrogens.

Bishop, Boycott and Zuckerman (7) studied the estrogenic properties of diethylstilbestrol on 46 women and 6 monkeys. They found its action similar to the natural estrogens. The following effects were noted.

(*a*), The production of 'estrin withdrawal bleeding.'
(*b*), The production of a proliferative endometrium both in the human female and in the monkey.
(*c*), Production of growth in a hypoplastic uterus.
(*d*), The relief of menopause symptoms.
(*e*), The conversion of a 'menopausal' into an 'estrous' type of vaginal smear.
(*f*), The relief of vulval atrophy resulting from the loss of ovarian function.
(*g*), The production of painful swelling of the breasts.
(*h*), The causing of proliferation and activation of the epithelium of the mammary glands.
(*i*), The relief of pain in dysmenorrhea.

They noted few toxic symptoms. Nausea and headache were experienced by one patient early during the treatment but not later. Vomiting occurred in 2 cases, while another patient took a total of 280 mg. by mouth during a 2-month period without any unpleasant by-effects.

Winterton and McGregor (8) treated a series of 45 cases with diethylstilbestrol. The complaints were endocrine disturbances such as postmenopausal syndrome, senile vaginitis, leukoplakic vulvitis, kraurosis vulvae, amenorrhea, dysmenorrhea, and 6 individuals in whom it was desirable to inhibit lactation. The responses obtained were similar to those which would have been expected from treatment with a natural estrogen. Moreover, oral administration of the synthetic substance was found to be as effective as intramuscular therapy. Slight nausea was occasionally experienced, but in only one case was the treatment poorly tolerated.

Loeser (9) found diethylstilbestrol a satisfactory substitute for ovarian follicular hormone in various forms of ovarian insufficiency. In 6 out of 15 cases side effects such as nausea and vomiting were produced.

Kreitmar and Siekmann (10) employed the acetate and propionate of diethylstilbestrol. They produced estrus in mice with the same dosage as estrone, but in rats the compounds were 5 times as effective by subcutaneous, and 20 times by oral administration. The duration of action is only prolonged with the esters. Antimasculine action was demonstrated in capons.

Buschbeck and Hausknecht (11) treated 90 women with various gynecological

disorders with diethylstilbestrol. Menstruation was produced in 4 cases of amenorrhea by 5×5 mg. of stilbestrol. Vaginal ulcers and pruritis vulvae cleared up. Menopausal disorders were ameliorated. The side actions were lassitude, feeling of illness and vomiting.

Ehrhardt et al. (12) found that 0.5 mg. of diethylstilbene dipropionate had an effect on the menopausal uterine mucous membrane equivalent to that of 1 mg. of estradiol monobenzoate. Twelve mg. of the stilbene preparation was required to transform the resting uterine mucosa (menopause, oöphorectomy) into the proliferative phase. Nausea, vomiting and frequency of micturition were observed if larger doses were given in one injection (3 to 5 mg.).

Engelhart (13) administered diethylstilbestrol (28 mg.) for 16 days to a woman 2 years after a double ovariectomy. The length of the uterus, determined by sound, was increased by 2.5 cm.; the uterine mucous membrane changed to the proliferative phase. Progesterone was given from the 16th to 23rd day (80 mg.); the secretory phase of the endometrium was produced. Menstruation occurred and colostrum could be expressed from the breasts.

Jacobsen (14) found that diethylstilbestrol abolished the histological castration signs in the anterior pituitary. It had twice the effect of estrone.

Dawson and Robson (15) reported that when rabbit's, guinea pig's, and frog's small intestine, and rabbit's, guinea pig's, and rat's uteri were suspended in oxygenated physiological solution, the addition of diethylstilbestrol in concentrations of $1/10^6$–$1/10^7$ produced: (*a*) inhibition of spontaneous rhythmic contractions and of tone; (*b*) inhibition of motor responses of the muscles to adrenalin, acetylcholine, histamine, posterior pituitary extract, $BaCl_2$, and direct stimulation. All these effects were slowly reversible. Estradiol and progesterone in concentrations of 1 in 2×10^5 produced an effect on the rabbit's small intestine in vitro similar to that produced by stilbestrol $1/10^6$. Intravenous injection of 10 to 20 mg. of stilbestrol produced only temporary and small effects on the blood pressure and on the intestinal and uterine movements recorded in situ.

Cobet and Ratschow (16) found that the acetate and propionate of diethylstilbestrol were beneficial in local circulatory derangements such as Raynaud's disease, coronary disease, circulatory disorders of the menopause and chronic arthritis.

Loeser (17) reported that the action of diethylstilbestrol was similar to that of follicular hormone except that intrauterine injection had no action on the thyroid. High dosage produced toxic changes in the liver and adrenals.

Palmer and Zuckerman (18) reported that diethylstilbestrol resembled natural estrogens in sensitizing the endometrium to progesterone action, in the production of estrous vaginal changes on local application to the vagina, and in percutaneous absorbtion.

Brühl (19) used both the acetate and propionate of diethylstilbestrol and found them more potent than follicular hormone. The acetate was not well tolerated; not more than 0.75 to 1.0 mg. should be administered in one dose.

Grab (20) compared the action of estradiol, estradiol benzoate with diethylstilbestrol, its acetate and propionate. The last two esters were 6 to 10 times as active as estradiol benzoate when given by mouth. The stilbene derivatives had the following effects. They changed the castration pituitary into a normal pituitary; pregnancy cells could be produced in the anterior pituitary by prolonged administration; the increased gonadotropic hormone content of the anterior of ovariectomized rats was decreased. The excretion of estrogenic substances in urine after the administration of stilbestrol began after the onset of estrus; the feces did not contain estrogenic substances.

Cobet et al. (21) noted that diethylstilbestrol, its dipropionate and diacetate protected normal and castrated male and female rats against tail necrosis produced by ergotamine or ergotamine and adrenalin injection in the same way as did the natural hormones. General symptoms after repeated administration included roughening of the fur, anorexia, apathy, conjunctivitis and priapism or vaginal opening.

Kellar and Sunderland (22) reported cases of menopausal syndrome, senile vaginitis, leucoplakia vulvae, inhibition of lactation and secondary amenorrhea treated with stilbestrol, which produced results comparable with those of a natural estrogen. The drug is active when given by mouth but may produce nausea.

Clinical Observations

We studied 40 gynecological cases. All were given diethylstilbestrol by mouth or by intramuscular injection. The dosages ranged from 0.05 mg. to 1 mg. once a day by mouth to 5 mg. twice a week by injection.

There were 15 menopause cases. They included patients with spontaneous, surgical and radiation menopause. Their ages ranged from 39 to 65 years. The symptoms complained of were the usual vasomotor disturbances and some those of vulval atrophy. The results obtained were as follows.

(*a*), All patients obtained symptomatic relief.

(*b*), The improvement frequently began 12 hours after the initial dose whether it was given by mouth or by intramuscular injection.

(*c*), In 10 cases the treatment with diethylstilbestrol had to be discontinued because of persistent nausea and often vomiting; 1 mg. per day per os was sufficient to produce constant nausea in some patients. It did not matter, so far as the nausea was concerned, as to the time of day the medication was taken or as to food relationship. In 2 patients severe epigastric symptoms occurred, simulating those of gastric ulcer. The patients in this group, 10, stated that they would rather have their original (menopause) symptoms than the persistent nausea.

(*d*), In the 5 remaining cases nausea was not complained of, or the symptom was so slight that the patients were not disturbed by it.

(*e*), Diethylstilbestrol changed the vaginal smear from that of the castrate to that of definite follicular activity. No difference could be observed in the character of the smears between the hormonal estrogens and the stilbene derivatives.

(*f*), Diethylstilbestrol caused the disappearance of follicle stimulating hormone from castrate urine.

(*g*), It also caused a lowering of the PH of the vagina.

One other observation appears to be of considerable importance in the use of diethylstilbestrol. Menopause patients treated with adequate amounts of the hormonal estrogens experienced, aside from the relief of symptoms, a very definite sense of well being. This was completely lacking when the patient was taking the stilbene derivatives. It is true that the vaginal smear, the PH, and the follicle stimulating hormone in the urine, changed in the favorable direction more rapidly with the diethylstilbestrol than with estradiol or its derivatives, but this did not compensate the patient for being constantly nauseated and wretched. We feel that diethylstilbestrol is only a fair and incomplete substitute for the hormonal estrogens and do not advocate the substitution of this compound for the natural estrogens. It is quite possible that other derivatives of stilbene will equal or exceed all the beneficial effects of the hormonal estrogens.

The origin of the nausea and vomiting is of great interest. The vomiting is not projectile in character but seems to be secondary to the severe nausea. The nausea

may be due to a local action within the stomach, such as could be produced by excretion of the drug through the stomach. This would also explain the severe pain, simulating a gastric ulcer, experienced by 3 patients. Why some patients experience little or no nausea with the same dosage is not as yet clear. The metabolism of the stilbene compounds may differ in these patients.

The remaining 25 cases presented symptoms such as amenorrhea, oligomenorrhea, dysmenorrhea or genital hypoplasia. The therapeutic effects were no different from those obtained with the hormonal estrogens (aside from the unpleasant by-effects in 16). Several cases are worthy of particular mention.

G.H., aged 24 years, complained of secondary amenorrhea of 2 years duration following induced abortion. No improvement was obtained with pregnancy mare serum (9 cc.). An endometrial biopsy failed to reveal any endometrium. The patient was injected with 20 mg. of diethylstilbestrol (5 mg. on 4/20, 4/27, 5/4, 5/11). On 5/16 the patient began a period which lasted 7 days. Nausea occurred only during the flow. On 5/25 the patient was injected with 5 mg. of stilbestrol and an intrauterine balloon was inserted. Minute rapid contractions were obtained. Ten mg. was injected on 6/1/39. The patient began to bleed on 6/7 and this terminated on 6/13. Nausea occurred during the flow only. An intrauterine balloon was inserted on 6/8, the second day of the flow. Minute contractions were obtained. An endometrial biopsy taken at the same time revealed a proliferative endometrium.

In a previous communication we (23) pointed out that the uteri of patients having long periods of amenorrhea fail to reveal any spontaneous contractions on the recording kymograph. When estrogens are injected contractions appear and are always small in amplitude and fairly rapid. Hence diethylstilbestrol produces contractions in the human uterus in vivo similar to those produced by the hormonal estrogens.

We have previously reported that large doses of hormonal estrogens when administered during the first half of the menstrual cycle have a tendency to make the menstrual period come too early or too late. We are unable to predict the time of the next period in any such patients or of any two successive periods. In addition functional dysmenorrhea is frequently relieved by such therapy. Diethylstilbestrol possesses the same property in this regard as do the hormonal estrogens. This can be seen from the following 2 cases.

F.B., aged 28, came to us because of dysmenorrhea. She had a regular 26 to 30-day cycle, moderate flow with severe pain. Results of the pelvic examination were negative; gravida 0. The patient was given 5 mg. of stilbestrol by injection on 7th and 14th days of the cycle; the interval was 24 days. Moderate flow without pain occurred. There was a severe nausea and vomiting 7 hours after each injection. During the following cycle the patient was given 5 mg. of diethylstilbestrol on the 4th and 11th day of the cycle and the period recurred after an 18-day interval. The flow was moderate without pain. Nausea and vomiting occurred 7 hours after each injection.

V.M., came to us also because of dysmenorrhea. The pelvis was normal. She had 24-day cycles, moderate in amount. The patient was given 5 mg. of diethylstilbestrol on the 7th and 14th day of the cycle. The interval was 43 days. There was no pain and a moderate flow. Biopsy on the 42nd day of the cycle revealed a late secretory endometrium. There were no unpleasant symptoms following the injections.

SUMMARY

Diethylstilbestrol when administered to women possessed the same physiological properties as do the hormonal estrogens. The therapeutic use of diethylstilbestrol is greatly limited because of the persistent nausea and occasional vomiting that was induced in more than one-half of the patients.

REFERENCES

1. Dodds, E. C., and W. Lawson: *Nature* 139: 1068. 1937.
2. Dodds, E. C., L. Goldberg, W. Lawson and R. Robinson: *Nature* 141: 247. 1938; 142: 34. 1938.
3. Dodds, E. C., W. Lawson and R. L. Noble: *Lancet* 1: 1389. 1938.
4. Noble, R. L.: *Lancet* 2: 192. 1938; *J. Physiol.* 94: 177. 1938.
5. Parkes, A. S., E. C. Dodds and R. L. Noble: *Brit. M. J.* 2: 557. 1938.
6. Folley, S. J., and H. M. S. Watson: *Lancet* 2: 423. 1928.
7. Bishop, P. M. F., M. Boycott and S. Zuckerman: *Lancet* 1: 5. 1939.
8. Winterton, W. R., and T. N. MacGregor: *Brit. M. J.* 1: 10. 1939.
9. Loeser, A. A.: *Brit. M. J.* 1: 13. 1939.
10. Kreitmar, H., and W. Siekmann: Klin. Woch. 18: 156. 1939.
11. Buschbeck, H., and K. Hausknecht: *Klin. Wchnschr.* 18: 160. 1939.
12. Ehrhardt, K., H. Kramann and H. Schäfer: *Munch. Med. Wchnschr.* 86: 261. 1939.
13. Engelhart, E.: *Wien. Klin. Wchnschr.* 51: 1356. 1938.
14. Jacobsen, E.: *Endokrinol.* 21: 20. 1938.
15. Dawson, R. F., and J. M. Robson: *J. Physiol.* 95: 420. 1939.
16. Cobet, R., and M. Ratschow: *Klin. Wchnschr.* 18: 347. 1939.
17. Loeser, A.: *Klin. Wchnschr.* 18: 346. 1939.
18. Palmer, A., and S. Zuckerman: *Lancet* 236: 933. 1939.
19. Brühl, R.: *Munch. Med. Wchnschr.* 86: 582. 1939.
20. Grab, W.: *Munch. Med. Wchnschr.* 86: 346. 1939.
21. Cobet, R., M. Ratschow and M. L. Steckner: *Klin. Wchnschr.* 18: 278. 1939.
22. Kellar, R. J., and J. K. Sutherland: *J. Obst. & Gynec.* 46: 1. 1939.
23. Wilson, L., and R. Kurzrok: *Endocrinology* 23: 79. 1938.

THE ACTION OF ESTRADIOL, PROGESTERONE AND TESTOSTERONE ON THE CONTRACTIONS OF THE HUMAN UTERUS

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IN A PREVIOUS publication the contractility of the intact human uterus during the normal menstrual cycle and in various functional menstrual disorders was described (1). The contractions were recorded by the intra-uterine balloon method. It was demonstrated that during the follicular phase the contractions are of small amplitude, short duration and frequent occurrence while in the luteal phase they are large, prolonged and less frequent. It was possible to reproduce the myometrial cycle in a woman (whose ovaries were not functioning) by administering the appropriate ovarian hormones in proper sequence.

The present report deals with further studies which indicate that progesterone in the absence of estrogenic hormone has little effect on the human myometrium while testosterone alone results in contractions which resemble those seen after simultaneous administration of estradiol and progesterone.

Case Report. M.M., aged 37 years, married, never pregnant, attended the Vanderbilt Clinic during the past five years for treatment of primary amenorrhea. No subjective symptoms other than absence of menstruation were reported. The breasts and external genitalia were somewhat hypoplastic; the uterus was anteverted, freely movable and slightly small. The adnexae were not palpable. No obesity or hirsutism was present. Basal metabolism ranged from -3 to -23%. Repeated examination revealed absence of both estrogenic and gonadotropic hormones in the urine, atrophy of the endometrium and absence of uterine contractions. These findings were interpreted as indicating failure of ovarian and pituitary gonadotropic function. As previously reported (1), the administration of estrogenic hormone (estradiol benzoate) resulted in the small rapid follicular-phase type of contraction (fig. 1a) while the simultaneous injection of estrogen and progesterone evoked the large slow contractions seen during the luteal phase of the cycle (fig. 1b).

A. Effect of male sex hormone. After a suitable interval to permit elimination of previously injected hormones during which the contractions completely disappear, the administration of testosterone propionate[1] was begun. Intramuscular injections of 50 mg. were given semi-weekly. The earliest effect on the myometrium was noted in about 10 days. Figure 2a indicates uterine motility after 3 weeks therapy (200 mg.) and figure 2b after 6 weeks treatment (500 mg.). At this point the injections were stopped. Ten days later large contractions were still present (fig. 2c) and the myometrium gave a characteristic response to 10 U of pituitrin injected intramuscularly (fig. 2d). Spontaneous motility continued actively for one month after discontinuation of testosterone therapy and gradually subsided during the second month. After this little or no activity was noted. The resemblance of the testosterone contractions to those present during the luteal phase of the menstrual cycle is striking.

B. Effect of progesterone alone. The administration of progesterone was started 4 months

[1] Perandren, Ciba.

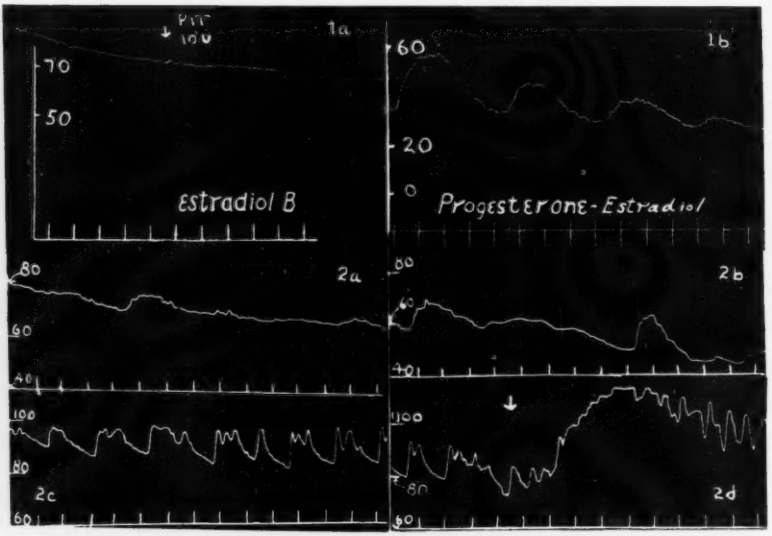

Fig. 1a. Contractions resulting from administration of estradiol benzoate alone. (All observations have been made on the human uterus in vivo.) Fig. 1b. Contractions resulting from simultaneous administration of estradiol benzoate and progesterone. Fig. 2a. Contractions resulting from administration of 200 mg. testosterone propionate within 3 weeks. Fig. 2b. Contractions after 500 mg. testosterone propionate within 6 weeks. Fig. 2c. Contractions 10 days after cessation of testosterone administration. Fig. 2d. Response to pituitrin (10 u).

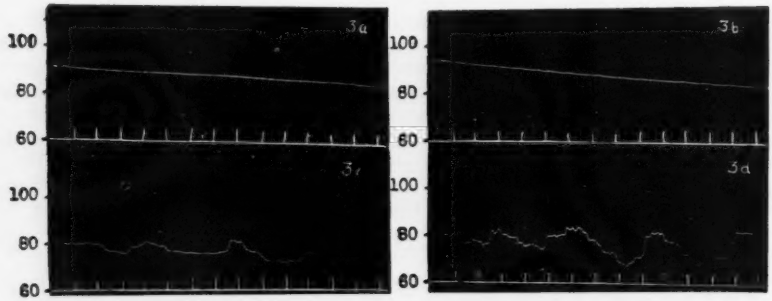

Fig. 3a. Absence of uterine contracility 4 months after last testosterone injection. Fig. 3b. Practically no effect after 75 mg. progesterone within 4 weeks. Fig. 3c. Contractions after 2 weeks of combined estrogen-progesterone therapy. Fig. 3d. Contractions after 3 weeks of combined estrogen-progesterone therapy.

after the last testosterone injection. At this time myometrial activity had ceased completely (fig. 3a). Intramuscular injections of 10 mg. of progesterone[2] were given semi-weekly. Very little effect was noted after a month of treatment (75 mg.) (fig. 3b). Some minute waves appeared from time to time but they were not true contractions. These waves were caused by slight but progressive decreases in tonus. The administration of pituitrin produced a slight tonus rise followed by small but somewhat prolonged contractions.

[2] Proluton, Schering, and progestin, Roche-Organon.

C. Effect of progesterone plus estrogen. At this point progesterone (10 mg.) together with estradiol benzoate[3] (1.66 mg.) were administered intramuscularly twice weekly. Two weeks later vaginal bleeding appeared. The flow lasted 4 days, was moderate in amount and was accompanied by abdominal cramps resembling dysmenorrhea. Kymographic study on the second day of this period revealed moderately large and prolonged contractions (fig. 3c) similar to those seen in the luteal phase of the menstrual cycle. Endometrial biopsy was attempted at this time but no tissue was obtained. One week later, with the combined estrogen-progesterone treatment maintained at the previous dosage, bleeding recurred. The flow again was moderate, lasted 4 days and was accompanied by abdominal cramps. The uterine contractions recorded on the second day of this flow were also large and prolonged (fig. 3d). At this point, with no change in dosage, a progessive decrease in amplitude occurred and it was not until 2 weeks later that the large contractions reappeared.

Because of obvious limitations it has not been possible to perform these studies on a large number of women. We have, however, repeated these studies in 2 cases of secondary amenorrhea and in one case of spontaneous menopause, with substantially the same results.

DISCUSSION

It is thus seen that the well-known 'one-two' reaction of estrogen and progestin on the endometrium (2) applies equally to the human myometrium. Progesterone, in the dosage employed, has no appreciable myometrial influence but acts only in the presence of estrogenic hormone. On the contrary, testosterone alone produces a type of uterine motility which resembles that seen during combined estrogen-progesterone administration and during the luteal phase of the menstrual cycle. The exact explanation for this action of testosterone is not known but may be accounted for by its close chemical and biological relation to both progesterone and the estrogens.

What is the significance of the androgens present in the urine of normal women? Are they merely useless by-products in the metabolism of the female sex hormones or do they play some physiological rôle? Too little is known at present to suggest an answer to these interesting questions.

The prolonged effect of testosterone propionate on the human myometrium after its administration has been stopped suggests that the hormone is either retained in the body for a long time or that actual morphological changes in the muscle fibers have occurred and that these take considerable time to regress.

The failure to maintain the large type of contraction despite continued estradiol-progesterone administration suggests the possible existence of some cyclic mechanism which prevents constant myometrial stimulation.

SUMMARY

Progesterone in the absence of estrogenic hormone has little effect on the human myometrium. Testosterone propionate alone produces uterine contractions resembling those seen after combined estrogen-progesterone treatment and simulate those of the luteal phase of the menstrual cycle.

We are indebted to Professor Benjamin P. Watson for encouragement and advice, and for placing the large material of the Sloane Hospital for Women and the Vanderbilt Clinic at our disposal. We also wish to thank Miss Belle Francis for her constant cooperation and tactful handling of our patients. We wish to thank Professor Charles C. Lieb for the use of his kymograph.

REFERENCES

1. Wilson, L., and R. Kurzrok: *Endocrinology* 23: 79. 1938.
2. Hisaw, F. L., H. L. Fevold and R. K. Meyer: *Anat. Rec.* 47: 300. 1930.

[3] Progynon-B, Schering, 1.66 mg. = 10,000 R.U.

SEX HORMONE STUDIES IN MALE HOMOSEXUALITY

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UNTIL RECENT YEARS homosexuality has been quite generally considered to be of psychogenic origin. But many new investigations cast doubt on this concept. A better insight into the mechanism of sexual development and sex differentiation points to the essential bisexuality of man. That sex anomalies may arise from abnormal genetic processes is substantiated by many observations on laboratory animals. Rosanoff (1) has emphasized the hereditary origin of homosexuality and other sexual anomalies in his theory of chaotic sexuality. Wright (2) postulated a theory of

TABLE 1. ANDROGEN–ESTROGEN RATIOS OF NORMAL SUBJECTS

Laboratory code No.	Androgens, I.U.	Estrogens, γ	Ratio	Laboratory code No.	Androgens, I.U.	Estrogens, γ	Ratio
51	24.3	1.50	16.22	70	33.6	2.40	14.00
52	27.4	4.50	6.10	71	37.5	15.00	2.50
53	26.6	1.50	17.75	72	32.7	1.50	21.79
54	37.0	1.50	24.65	107	16.4	1.50	10.93
55	22.6	3.00	7.53	123	36.4	7.50	4.85
57	45.8	5.25	8.73	125	31.7	1.50	21.10
59	56.0	15.00	3.73	126	40.6	7.50	5.42
60	41.0	7.50	5.47	127	30.4	3.75	8.11
61	36.2	4.50	8.05	128	47.2	4.50	10.50
62	41.0	5.25	7.81	129	55.9	1.50	37.25
63	34.0	5.25	6.48	130	37.1	1.50	24.70
64	26.5	5.25	5.05	133	19.0	1.50	12.67
65	33.6	7.50	4.48	135	23.4	1.50	15.60
66	33.1	3.75	8.84	136	29.6	7.50	3.95
67	16.9	2.40	7.05				
68	45.8	3.00	15.27	Average Values	34.1	4.41	12.01
69	38.4	1.50	25.60				

homosexuality based on a sex hormone imbalance. He showed various degrees of reversal of the normal androgen-estrogen ratio in 49 homosexuals. Glass and McKennon (3) likewise demonstrated these differences but their data were too meager for statistical analysis.

It seems highly desirable to extend these exploratory studies with the more refined methods of assays now available, and to seek further evaluation of the sex hormone findings in homosexuality. If a biologic component can be demonstrated in the majority of congenital homosexuals then the clinical concept of this sex anomaly may need considerable revision. The growing importance of the sociological aspects of the subject makes urgent the continued investigation of the problem from a broad psychosomatic perspective.

This report is concerned with the sex hormone findings in 17 clinically diagnosed male homosexuals and 31 normal males as controls. Three other subjects taken into custody with the homosexual group were found on clinical study to be normal heterosexuals. This diagnosis was later substantiated by the finding of normal sex

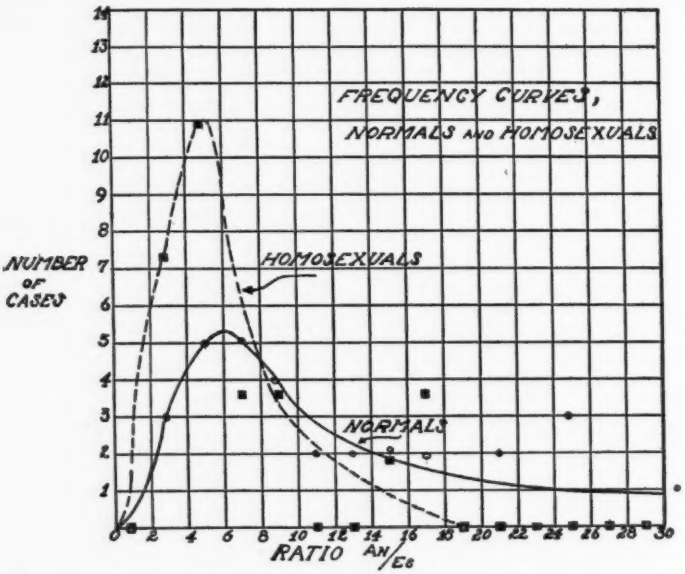
TABLE 2. ANDROGENS–ESTROGENS RATIOS OF HOMOSEXUAL SUBJECTS

Case No.	married or single	Age	Specimen	Androgens, I.U.	Estrogens, γ	Ratio	Average
1	M.	59	16	30.0	10.50	2.85	
			118	16.8	5.25	3.20	3.02
2	S.	31	14	55.5	4.50	12.33	
			109	27.0	7.50	3.60	
			134	27.4	5.25	5.21	7.05
3	S.	30	3	18.7	15.00	1.25	
			88	20.8	5.25	3.95	
			116	30.9	5.25	5.88	3.69
4	S.	51	17	50.7	7.50	6.76	
			103	12.9	7.50	1.72	4.24
5	M.	54	1	27.4	5.25	5.22	
			86	45.0	1.50	30.00	
			124	39.7	5.25	7.56	14.03
6	S.	18	8	34.0	3.00	11.33	
			84	20.8	5.25	3.95	
			112	21.7	18.00	1.20	5.49
7	S.	41	13	48.5	35.00	3.26	
			105	12.5	1.50	8.33	5.79
8	S.	23	9	42.8	3.00	14.27	
			85	37.5	10.50	3.57	8.92
9	S.	48	15	44.6	1.50	29.73	
			106	22.1	5.40	4.09	
			119	29.6	1.50	19.73	17.85
10	S.	24	18	50.7	10.50	4.83	
			113	21.7	1.50	14.47	9.65
11	S.	38	5	23.0	4.50	5.11	
			81	27.4	5.25	5.22	
			117	20.4	6.00	3.40	4.57
12	S.	24	10	23.0	4.50	5.11	
			87	42.8	10.50	4.07	
			120	32.7	16.50	1.98	3.03
13	S.	25	12	23.9	10.50	2.27	
			101	14.2	7.50	1.89	2.08
14	M.	52	11	36.2	10.50	3.45	
			114	12.0	1.50	8.00	5.72
15	S.	18	7	40.6	1.50	27.07	
			83	14.2	7.50	1.89	
			104	18.1	1.50	12.07	
			132	35.3	1.50	23.53	16.14
16	M.	42	19	31.4	7.50	4.19	
			110	19.5	2.50	7.80	6.00
17	S.	20	6	47.2	7.50	6.29	
			82	30.9	3.00	10.30	
			102	23.9	5.25	4.55	
			131	41.5	5.25	7.90	7.26
Average values				29.9	6.34		7.33

TABLE 3. SUMMARY AND COMPARISON OF RATIOS OF "KNOWN AND UNKNOWN" SPECIMENS

	Androgens, I.U.	Estrogens, γ	Ratio	Ratio Specimens by code 100 series only
17 Homosexuals	29.9	6.34	7.33	7.27
31 Normals	34.1	4.41	12.01	14.09

hormone values. The normal controls consisted of two separate groups. The first group was composed of 20 students ranging in age from 23 to 28 and the second consisted of 11 married men ranging in age from 18 to 62. A comprehensive clinical re-



port will follow the completion of this clinical study as well as on a trial of organotherapy in the homosexual group.

METHODS

The sex hormone ratios were determined by quantitative assays of the urinary androgens and estrogens. No attempt was made to separate the various estrogenic and androgenic compounds in the urine. The values expressed are in international androsterone units and in gamma of estrin per day. Our former observations (2, 3) were recorded simply in terms of capon and mouse units. The present report deals with the ratios of androgens and estrogens in each respective group, and the values represent international unitage.

The tests on the homosexuals were carried out on several series of urine specimens. The first represented a 5-day pooled collection obtained when the group was institutionalized. The second and third series of specimens were 7 night and first morning collections. Those pooled samples were considered as 56-hour specimens. The first group of normals submitted 48-hour total collections. The second group collected 7 night and first morning specimens.

The last series of tests on all the homosexuals and on the second control group were identified by code. Most of the pooled urine was preserved with toluol and refrigerated. The androgens were determined by two different existing methods of assay: (*a*) a modified Gallagher-Koch (6) capon technic and (*b*) the colorimetric test of Callow and Emmens (7), using a Klett-Summerson photoelectric colorimeter and a Wrattan filter #62. The standardization of pure androsterone solutions by this procedure gave results practically identical with those given by Callow and Emmens. Because of an inadequate number of capons necessary for the large number of tests and the relative ease and reliability of the colorimetric method, it was deemed advisable to carry out the majority of the androgen estimations by colorimetric determinations. The values are expressed as international androgenic (androsterone) units per day.

The estrogens were estimated by modifications of existing methods on spayed adult rats. The values are expressed as gamma of estrin per day.

The gonadotropic factor in the urine was determined by the Zondek alcohol precipitation method, using immature mice. Vaginal canalization, Graafian follicle stimulation plus cornification was taken as a positive response. The values are expressed in mouse units per day. Only the homosexual subjects were tested for this hormone.

RESULTS

Differences in the sex hormone ratios were demonstrated with repeated testing as shown in the three tables and as illustrated in the frequency distribution curve. The homosexual group shows ratios which are distinctly lower than those of the normal controls. It is clearly evident that the estrogenic values are higher among the homosexuals, whereas there are less striking differences in the androgenic values. Although there is considerable spread in values obtained in some of the subjects, it is noteworthy that the average ratios of the various series of assays show satisfactory agreement. The last batch of normal and homosexual specimens which were submitted under code numbers showed the same significant difference in hormone ratios.

The findings may be roughly summarized as showing that: (*a*) Two-thirds of the homosexuals had androgen values less than 30 I.U. and estrogen values greater than 5γ (33% had ratio values less than 6); (*b*) Two-thirds of the normals had androgen titers greater than 30 I.U. estrogens less than 5γ. The gonadotropic values among the homosexuals ranged from 0 to 50 M.U. This was not considered significant enough to warrant repeated testing.

In face of such highly suggestive hormonal differences one may assume that such data point to a definite biologic mechanism in homosexuality. Of course it is not possible at this time to evaluate the true significance of the difference, but it seems that the constitutional homosexual has a different sex hormone chemistry than the normal male. Only future research may properly assess this biologic component. However, enough evidence has been introduced thus far to warrant the use of sex hormone assays in diagnostic studies of homosexuality. Such objective study may differentiate between so-called acquired, latent and congenital (constitutional) homosexuality.

It is difficult to reconcile some of the discrepant results in this study, viz: the low ratios among normals and high ratios among homosexuals. Those few normal subjects may be latent homosexuals, whereas the homosexuals with the high ratios may not be of the true constitutional type. The greater consistency of the findings in the homosexual group undoubtedly indicates a better selection of the homosexuals than of the normals. It is evident, nevertheless, that the bulk of the data is highly concordant.

The relative agreement between the results of known and unknown specimens is also significant. Table 3 illustrates this very well. The homosexual ratios of 7.33 and 7.27 are almost identical. The slight difference in the normal ratios of 12.01 and 14.09 may be due to a better selection of the second group of normal subjects who were mostly mature married men.

Although the differences in the androgen: estrogen ratio between the homosexual and control group are statistically significant, it should be pointed out that wide variations were found in different assays on the same homosexual subject. Such discrepancies are concerned chiefly with alterations in the estrogen excretion. One must exercise caution, therefore, in attributing too wide significance to the result of individual determinations.

Such data suggest revision of our clinical concept of this widespread sexual anomaly. Obviously, if a biologic etiology were established, this would lead to investigation of therapeutic possibilities from a much wider perspective than now exists. Much more research is urgently needed to clarify the many questions facing further study of homosexuality.

SUMMARY

Sex hormone assays in 17 male homosexuals yielded androgen: estrogen ratios which appear to be significantly lower than those obtained in 31 normal males.

An advisory committee consisting of Professor B. M. Allen, Drs. George Dock, W. L. Halverson, V. Parkins, F. M. Pottenger and E. K. Shelton offered suggestions for the prosecution of this work and recommended publication of the data collected as an exploratory study.

Sex hormone assays were carried out in the hormone laboratory of Dr. S. J. Glass.

We are greatly indebted to M. Levi, Dr. H. Hess and Mr. Kritzer for technical assistance and to Dr. H. Blunden and Lois Hallman of the Department of Biochemistry, University of Southern California Medical School, for colorimetric determinations of androsterone.

The androsterone used in this study was kindly donated by the Ciba Pharmaceutical Products Co.

REFERENCES

1. Rosanoff, A. J.: *Am. J. Psychiat.* 92: 35. 1935.
2. Wright, C. A.: *Med. Record* 142: 407. 1935; 147: 449. 1938; 149: 399. 1939.
3. Glass, S. J., and B. J. McKennon: *West J. Surg.* 45: 467. 1937.
4. Gallagher, J. E., D. H. Peterson, R. I. Dorfman, A. T. Kenyon and F. C. Koch: *J. Clin. Investigation* 16: 695. 1937.
5. Callow, R. K., and C. W. Emmens: *Biochem. J.* 32: 1312. 1938.

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THE EFFECT OF ANTERIOR PITUITARY-LIKE SEX HORMONE ON THE BLOOD PICTURE IN MAN

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IN THE LITERATURE to date observations of the effect of gonadotropic hormone extracted from pregnancy urine on the blood picture are few, and no adequate study of such effect has been made in man. In 1931 Novak and Hurd (1) suggested that the results obtained from the administration of this hormone in functional uterine hemorrhage were possibly due to changes produced in the blood. In the same year Stockinger and Berchtold (2) reported a marked transient rise in the white blood counts 1 to 1½ hours after the injection of "100 units of prehormone" in which the neutrophils and lymphocytes participated equally and which occurred at the time of appearance of a local reaction. They were inclined to consider the leucocytosis as non-specific, which seems probable as a very crude extract was employed. Geist and Spiellman (3) gave 1 cc. of antuitrin-S to 6 patients 3 times weekly for 2 to 3 weeks. Blood counts made before the series of injections was begun and again after all the injections had been completed showed no significant change. Wilson (4) observed that injections of A P L hormone extracts caused a marked leucocytosis in normal virgin female rabbits within 5 to 8 hours, and maintained it at a relatively high level for 48 to 72 hours. The red cell count remained unaltered. Repeated injections over a long period and at short intervals became ineffectual in producing a leucocytosis. This he thought might have some bearing on Collip's theory of antihormone control. Since Wilson administered a standard dose of 100 R.U. to rabbits weighing between 4 and 8 pounds, this amount was relatively far in excess of doses given therapeutically to human patients. The experiment reported in this paper was undertaken to determine the effect of ordinarily administered therapeutic doses in man.

PROCEDURE

The procedure was as follows. Blood counts were taken between 4 and 6 in the afternoon and again between 9 and 10 the following morning and then follutein was injected in doses of 100 to 200 U using both the concentrated (1000 R.U. per cc.) and the weaker (100 R.U. per cc.) solutions. At first blood counts were repeated every 2 hours for 8 hours, and again at 24 hours. No rise in the white count was observed under 4 hours and at 4 hours the rise was usually slight. The maximum rise occurred sometimes at 6 and in other cases at 8 hours. In a few cases follutein was administered at 6 P.M. and counts made at 9 o'clock the following morning. These 15-hour observations were consistently lower than the 8-hour observations. Because of working hours in the laboratory no counts were made between the ninth and fifteenth hours following injection. As a control the same procedure was repeated with the glycerine and sterile water solution in which follutein is dissolved. After these preliminary observations, the following routine was adopted. Counts were made in the afternoon and again in the morning preceding injection and then at 6 and 24 hours following injection. The temperature was taken each time a blood count was made and the

patient was questioned as to local or general reactions following injection, and the site of injection was observed for evidence of local reaction.

RESULTS

Table 1 shows the white count before and after injection of the various doses and dilutions of follutein, and of the glycerine solution controls, with the number of

TABLE 1. AVERAGE WHITE COUNTS BEFORE AND AFTER INJECTIONS OF FOLLUTEIN AND OF GLYCERINE-SOLUTION CONTROL

		P.M. before	A.M. before	P.M. 6 hours after	A.M. 24 hours after	No. of individuals
Glycerine solution	0.5 to 1.0 cc.	101	100	116	100	12
Glycerine solution	2.0 to 4.0 cc.	95	100	111	102	11
Follutein 100 R.U.	(0.1 cc.)	100	100	130	106	11
Follutein 100 R.U.	(1.0 cc.)	98	100	151	101	9
Follutein 200 R.U.	(0.2 cc.)	105	100	161	111	10
Follutein 200 R.U.	(2.0 cc.)	103	100	185	134	7

The white counts are transformed into terms of A.M. before = 100

individuals in each group. In order to make comparisons easier the white counts are transformed into terms of the white count before injection equalling 100. At 6 hours there was a slight rise following the injection of the glycerine solution, a moderate rise after 100 U of follutein, and a marked rise after 200 U. The difference between the rise following injection of the weaker and stronger solutions would seem roughly to

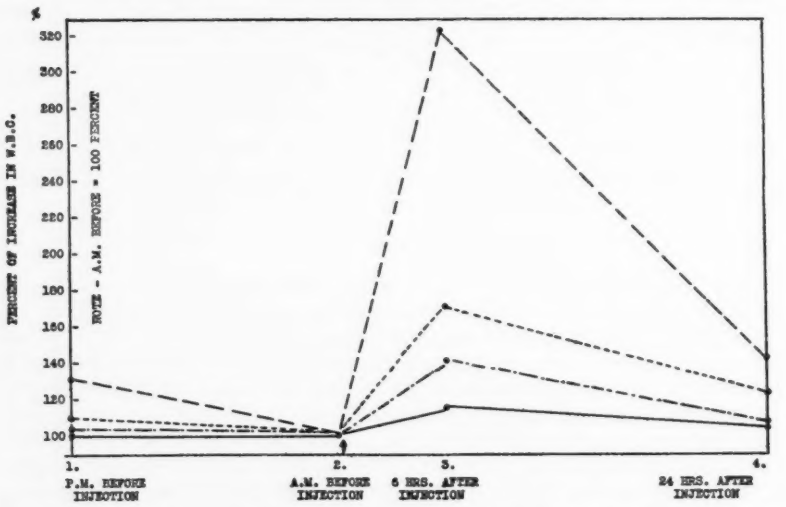

Fig. 1. COMPARATIVE EFFECTS OF GLYCERINE-SOLUTION CONTROL AND OF 100, 200 AND 400 R.U. OF FOLLUTEIN ON THE LEUCOCYTE COUNT. ARROW INDICATES TIME OF INJECTION.

be accounted for by the rise following injection of glycerine solution itself. At 24 hours after injection there was no rise following the glycerine solution, a very slight rise following 100 U of follutein, and a somewhat higher rise following 200 U.

Figure 1 shows a comparison of the effect of varying doses of follutein on the leucocyte count. The average is taken of all cases in which the same number of R.U. was administered without regard to dilution, and of all glycerine controls without regard to dosage. In order to show the percentage rise all counts are transformed into terms of the A. M. count immediately before injection equalling 100. Included is the one case in which a patient was given an initial dose of 400 R.U. (0.4 cc.) of follutein.

Table 2 shows the average differential white count before and at 6 hours after injection. In 20 glycerine controls with an average rise in white count of 15% there was no change in the differential count. In 10 subjects who after follutein showed a

TABLE 2. AVERAGE DIFFERENTIAL WHITE COUNT BEFORE AND AFTER INJECTION OF FOLLUTEIN AND OF GLYCERINE-SOLUTION CONTROL

	Neutrophils %		Lymphocytes %		Monocytes %		Eosinophils %		Basophils %		Nonsegmented neutrophils	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
In 20 glycerine controls with average rise in white count of 15%	62	62	30	31	5.6	4.6	1.9	2.2	0.2	0.4	2.7	2.0
In 10 subjects with an average rise in white count after follutein of 35%	61	68	30	26	6.7	4.1	2.4	1.3	0.1	0.3	2.8	3.1
In 10 subjects with an average rise in white count after follutein of 221%	61	78	29	21	6.5	4.5	2.9	1.5	0.1	0	1.3	6.7

comparatively low rise in white count with an average of 35% there was a slight increase in percentage of neutrophils and decrease in percentage of lymphocytes. In the 10 subjects who showed the greatest rise in white count averaging 221% there was a more marked increase in percentage of neutrophils and decrease in percentage of lymphocytes. Other types of white cells showed no significant change. The average rise in percentage of non-segmented neutrophils in the subjects with the greatest rise in white count was due to 2 patients, in one of whom the non-segmented neutrophils rose from 0 to 15%, and in the other from 7 to 30%. Incidentally, in both of these patients the rise in white count was below the average of the group.

When the absolute number of cells is considered rather than percentage it appears that there was an increase in white cells of all types in the circulating blood following the administration of follutein.

When the same dose of either 100 or 200 R.U. was injected daily the leucocytosis gradually diminished and in most patients disappeared after the seventh day. After an interval of 2 or 3 weeks it could again be elicited. Also a rise followed any marked increase in the daily dose and after daily administration of 400 to 500 R.U. there usually was still a significant rise even after 2 weeks. Follutein was administered to 8 males and 30 females. No difference in effect on the basis of sex was observed. There was no change in the red cell count nor in hemoglobin at any time, after either single or daily repeated injections of follutein.

DISCUSSION

According to the manufacturer: "follutein is protein-free but contains a small amount of polypeptides. The total amount of nitrogen per unit is about one-tenth that in a unit of insulin." It seems unlikely that this could account for the results observed. The possibility that the leucocytosis is of a toxic nature must be considered although there is considerable evidence against this. In the first place there was no correlation whatever between the height of the rise in white count and either general or local reactions or changes in the patient's temperature. In fact in many cases in which occurred the highest leucocytosis there was no reaction reported or observable and no rise in temperature. On the other hand, just as many of those patients who had reactions, showed only a slight or moderate rise in white count as a marked rise. Other evidence against the probability of a toxic reaction is the long latent period following injection and the absence, expect in 2 of a total of 38 patients, of an increase in percentage of non-segmented neutrophils.

Although there was an increase in the absolute number of all types of white blood cells, the relative neutrophilia might indicate that gonadotropic hormone extracted from pregnancy urine is a stimulus to bone marrow rather than lymphoid tissue.

SUMMARY

Gonadotropic hormone extracted from pregnancy urine (follutein) was administered intramuscularly to 38 individuals, 8 males and 30 females, in doses ranging from 100 to 400 R.U. Complete blood counts were made in the afternoon and again the next morning immediately preceding injection, and then at 2, 4, 6, 8, 15 and 24 hours following injection. A leucocytosis appeared at 4 hours, reached its greatest height at 6 or 8 hours, and then diminished but did not disappear at 24 hours. The degree of leucocytosis was roughly proportional to the amount of follutein given. There was an increase in the absolute number of white cells of all types with a relative neutrophilia. Following daily repeated doses the leucocytosis gradually diminished and eventually disappeared but after an interval of 2 to 3 weeks could again be elicited. The leucocytosis did not seem to be a protein or toxic reaction. The same effects were observed in both sexes. There was no change in the red cell count or in hemoglobin.

I am indebted to Dr. Horace Gray for his assistance in correlating the results here reported.

Both the follutein and the glycerine control solution used in this study were supplied by E. R. Squibb & Sons through the courtesy of Dr. J. A. Morrell.

REFERENCES

1. Novak, E., and C. B. Hurd: *Am. Gynec. Soc.* 56: 146. 1931.
2. Stockinger, W., and F. Berchtold: *Ztschr. f. d. ges. exper. Med.* 75: 827. 1931.
3. Geist, S. H., and F. Spiellman: *Proc. Soc. Exper. Biol. & Med.* 32: 353. 1934.
4. Wilson, D.: *Endocrinology* 21: 96. 1937.

ENDOCRINE DWARFISM

FOURTH REPORT

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SINCE OUR ORIGINAL STUDY, instituted with the late William Engelbach in 1932, some fifty patients displaying classical signs of pituitary dwarfism have been seen by the present senior author. Of this group 18 have been observed and treated for periods of from 10 months to 6 years and 4 months. Clinical observation in respect to dwarfism presents many pitfalls to the physician. Human growth is a variable with no great constancy in relation to individuals or particular to age level. The rate of growth in the dwarf is even more complex since all standards for man are a composite of variables and these standards cannot be applied to the dwarf. Therefore it follows that the rate of future growth in the untreated dwarf cannot be predicted, although, certainly, this would be below any accepted normal standard.

No final conclusions are drawn, but to entirely negate attempts to evaluate this

TABLE 1. ORIGINAL GROUP, DWARFISM

	Age	Period of time treated	Actual growth	Normal increment
R. S.	7 yr. 10 mo. to 12 yr. 6 mo.	4 yr. 8 mo.	9.8 in.	10.1 in.
C. M.	9 yr. 7 mo. to 15 yr. 11 mo.	6 yr. 4 mo.	14.0 in.	11.0 in.
P. G.	11 yr. 3 mo. to 16 yr. 2 mo.	4 yr. 11 mo.	15.7 in.	8.5 in.
D. T.	18 yr. 11 mo. to 20 yr. 8 mo.	1 yr. 9 mo.	6.2 in.	0.5 in.
Total			45.7 in.	30. 1 in.

problem clinically would seem illogical. Given a patient who fulfills the concept of pituitary dwarfism to whom a remedial agent is administered with resultant growth over the normal increment, it would seem significant, at least, that the medicament employed is a true growth stimulant. Given a group of 18 patients who fall into this classification and who have been adequately treated with normal growth resulting in 15, it would seem apparent that suggestive clinical deductions may be made.

That the eosinophilic cell of the hypophysis elaborates a factor, or hormone, which is necessary to human growth is undoubted. In the same instance we must realize, also, that there are other determining factors of human growth such as vitamins, food intake, environment and heredity.

Only those patients presenting proportionate statural underdevelopment were included in this series. None displayed any structural stigmata of primary hypothyroidism. In the original group 7 patients were treated during a control period of 2 to 8 months with a full, tolerant dose of desiccated thyroid. None attained any growth with the exception of one patient who grew 0.5 in. in 5 months, tending to prove that thyroid alone was not a growth stimulant in these instances. The method of treatment following this has been with the original pituitary growth factor[1] as prepared by Putnam, Teel and Benedict (1) in collaboration with Bugbee, Simond and

[1] Antuitrin-G, Parke, Davis & Company.

Grimes (2), although there have been employed many successive refinements and increased concentration in the extract. In addition, all patients were treated with a full, therapeutic dose of desiccated thyroid. This at first was empirical. Later its basis was demonstrated by Smith (3) in the experimental animal, revealing that a combination of thyroid with the pituitary growth factor enhanced the growth of the experimental

Fig. 1. A, P. G. before treatment, 11 yr. 2 mo. of age, 3/5/32; head, 20.7 (21.1 opt.); chest, 23.5 (26.1 opt.); abdomen, 22.0 (22.3 opt.); height, 47.5 (53.3–57.1); upper, 23.5 (26.1–28.7); lower, 23.2 (26.3–28.9); span, 46.0 (53.5–57.7); weight, 50.7 (63.0–79.4).

B, P. G. after treatment, 16 yr. 2 mo. of age, 3/1/37; head, 22.0 (22.0 opt.); chest, 32.0 (31.7 opt.); abdomen, 27.0 (27.2 opt.); height, 63.2 (61.8–66.2); upper, 30.2 (30.0–33.0); lower, 33.0 (31.0–34.0); span, 63.0 (63.4–68.2); weight, 117.0 (96.0–122.0). Period of time treated, 4 yr. 11 mo. Actual growth of 15.7 in., 7.2 in. over normal increment for age and time treated. Treatment was discontinued because normal height was attained and adolescence was beginning. Further growth can still be predicted as epiphyseal closure has not been completed. This patient had a generalized chondro-epiphysitis which also cleared entirely (5).

hypophysectomized animal as compared with the animal treated with the pituitary growth factor alone.

Four patients of the original group were treated and observed for periods of from 1 yr. 9 mo. to 6 yr. 4 mo. The age range was from 7 yr. 10 mo. to 18 yr. 11 mo. Patient 1 (R. S.) who displayed signs of infantilism, is the only one in this group whose actual growth has not exceeded the normal increment while on treatment. As yet, there are no signs of beginning adolescence, and because of her present age

TABLE 2. GROUP 2, DWARFISM

	Age	Period of time treated	Actual growth	Normal increment
D. B.	3 yr. 3 mo. to 4 yr. 9 mo.	1 yr. 6 mo.	1.8 in.	4.0 in.
J. C.	4 yr. 9 mo. to 7 yr. 1 mo.	2 yr. 6 mo.	7.2 in.	5.5 in.
	7 yr. 8 mo. to 7 yr. 10 mo.			
L. W.	6 yr. to 8 yr. 8 mo.	2 yr. 8 mo.	5.5 in.	5.4 in.
J. B.	9 yr. 3 mo. to 10 yr. 9 mo.	1 yr. 6 mo.	2.4 in.	3.0 in.
B. B.	12 yr. 6 mo. to 14 yr.	1 yr. 6 mo.	2.4 in.	2.7 in.
M. P.	13 yr. 10 mo. to 15 yr. 9 mo.	1 yr. 11 mo.	4.6 in.	1.8 in.
J. D.	15 yr. 4 mo. to 16 yr. 8 mo.	1 yr. 4 mo.	2.1 in.	1.9 in.
Total			26.0 in.	24.3 in.

(14 yr.) it has been advised that the growth hormone be withdrawn and that she be treated with the sex hormones to promote sexual development. Treatment was recently discontinued in *case 2*, C. M., because she was approaching normal statural height, and adolescence, which was beginning, should be permitted to occur. Treatment was discontinued in *case 3*, P. G., because his height came within the normal range. Adolescence was occurring, and as epiphyseal closure was not complete even further statural growth could be predicted. Treatment of *case 4*, D. T., was discontinued after 1 yr. 9 mo. because epiphyseal closure was being effected.

It is the writers' contention that there is a direct antagonism between the growth and sex factors of the pituitary. We believe that it is much better to have a moderately dwarfed individual with normal sex function than to have a normal sized or larger than normal, but under-sexed individual as the end result of treatment. Considerable clinical judgment must be employed in advising treatment approaching the time of adolescence. The outstanding determining clinical factors are epiphyseal closure and the development of secondary sex characteristics.

The original extract employed undoubtedly contained other pituitary factors. Clinical evidence of this was the general increase of the basal metablic rates in all instances. In 14 patients the average B.M.R. before treatment was +12% whereas it was +37% during treatment (4). No patient, however, displayed signs of clinical thyrotoxicosis. At that time no bioassay was made for the thyrotropic factor.

Seven patients composing the second group ranged in age from 3 yr. 3 mo. to 15 yr. 4 mo. Four showed a growth exceeding that of the normal increment for age and period of time treated. The remaining 3, similar to *case 1* in the original group, displayed signs of infantilism in addition to their pituitary dwarfism, and their growth did not exceed the normal increment while on treatment. During the first

TABLE 3. GROUP 3, DWARFISM

	Age	Period of time treated	Actual growth	Normal increment
R. P.	5 yr. to 7 yr.	2 yr.	4.6 in.	4.5 in.
E. R.	8 yr. 6 mo. to 9 yr. 11 mo.	1 yr. 10 mo.	4.7 in.	3.6 in.
	10 yr. 7 mo. to 11 yr.			
K. L.	10 yr. 2 mo. to 11 yr. 5 mo.	1 yr. 3 mo.	4.1 in.	2.9 in.
H. L.	10 yr. 2 mo. to 11 yr. 5 mo.	1 yr. 3 mo.	4.2 in.	2.9 in.
H. L.	10 yr. 9 mo. to 11 yr. 7 mo.	10 mo.	3.7 in.	1.6 in.
F. O.	16 yr. 8 mo. to 18 yr.	1 yr. 4 mo.	1.2 in.	0.4 in.
T. S.	17 yr. 8 mo. to 18 yr. 6 mo.	10 mo.	0.3 in.	0.06 in.
Total			22.8 in.	15.96 in.

TABLE 4. WITHDRAWAL OF MEDICATION, DWARFISM

	On treatment			Off treatment		
		Actual growth	Normal increment		Actual growth	Normal increment
D. B.	18 mo.	1.8 in.	4.0 in.	17 mo.	1.7 in.	3.3 in.
J. C.	30 mo.	7.2 in.	5.5 in.	15 mo.	2.5 in.	2.2 in.
J. B.	18 mo.	2.4 in.	3.0 in.	17 mo.	1.5 in.	2.7 in.
B. B.	18 mo.	2.4 in.	2.7 in.	17 mo.	1.3 in.	2.3 in.
M. P.	23 mo.	4.6 in.	1.8 in.	8 mo.	0.1 in.	0.4 in.
J. D.	16 mo.	2.1 in.	1.9 in.	26 mo.	5.4 in.	2.3 in.
E. R.	22 mo.	4.7 in.	3.6 in.	6 mo.	0.0 in.	1.0 in.
F. O.	16 mo.	1.2 in.	0.4 in.	7 mo.	0.0 in.	0.0 in.
	161 mo.	26.4 in.	22.9 in.	113 mo.	12.5 in.	14.2 in.
Average	20.1 mo. per patient			14.1 mo. per patient		

year of their treatment the extract undoubtedly contained some of the other pituitary factors as in group 1.

Group 3 also contained 7 patients, and all showed an actual growth exceeding the normal increment for age and the period of time treated.

While rather a poor parallel to the exact laboratory control method of study with the experimental animal, 8 of the total number of patients were taken off treatment for varying periods of time (from 6 to 26 mo.), and their growth was compared. The time of actual treatment was an average of 20.1 mo. per patient, and their total actual growth during this period was 26.4 in. The total normal increment for this time and age was 22.9 in. While off treatment, an average of 14.1 mo. per patient, their total actual growth was 12.5 in., and the normal increment for time and age was 14.2 in. While on treatment 5 patients exceeded the normal increment whereas during the withdrawal period only 2 continued to grow at the same comparative pace. It is significant that these patients did grow more individually and totally during the period of time treated than during the period of withdrawal.

A total of 18 patients were treated for a period of from 10 mo. to 6 yr. 4 mo. The total normal increment for age and period of time treated was 70.36 in. as compared with the actual growth of 94.5 in. attained. In other words, the total growth did actually exceed that of the increment for the normally growing child during this time. This is more remarkable when we consider the fact that previous to treatment their growth in no wise approached the normal rate. It is this finding which gives the determining factor in concluding that the material employed is a definite human growth stimulant. In our third report (4) the average actual gain in height on treatment of the 4 patients in the original group was 7.1 in. for an average period of 2.3 years. The average assumed growth for the previous 2.3 years prior to treatment was 2.0 in. The average normal increment for age and time was 4.0 in. Similarly in the second group the average actual gain in height on treatment was 2.2 in. for an

TABLE 5. TOTAL GROWTH, DWARFISM

No. Patients		Actual growth	Normal increment
4	Group 1	45.7 in.	30.1 in.
7	Group 2	26.0 in.	24.3 in.
7	Group 3	22.8 in.	15.96 in.
		94.5 in.	70.36 in.

averaged period of 11.7 mo. The average assumed growth for the previous 11.7 mo. prior to treatment was 0.5 in. The average normal increment for age and time was 1.9 in.

DISCUSSION

Eighteen patients showing signs of pituitary dwarfism were treated and observed over a period of from 10 mo. to 6 yr. 4 mo. All but 4 showed a growth exceeding that of the normal increment for the individual age of the patient and the period of time treated. These 4 patients, in addition to showing signs of pituitary dwarfism, also displayed signs of infantilism. This suggests the possibility that an admixture of the pituitary factors might prove to be more effective in their cases.

The average dose employed was 5 cc. of the extract given intramuscularly 2 times a week. In addition, a full tolerant dose of desiccated thyroid was given. No local or systemic reactions were noted in its administration during the first 2 years although 2 patients developed minor abscesses at the site of injection. With the higher concentration and purification of the extract in the past year we have seen several local and systemic foreign protein-like reactions.

We are not of the opinion that the ultimate therapy for human dwarfism has as yet been formulated. Better diagnostic criteria will aid greatly. We do believe that the material employed did have a definite growth stimulating effect. No untoward effects occurred in any of the patients except the minor reactions as cited. We feel at the present time that this medication is indicated when one is confronted with true human pituitary dwarfism. The major objection to this form of treatment is the fact that it takes some degree of heroism on the part of the young patient to subject himself to bi-weekly injections over a period of years. This was the most frequent reason for the inability to observe a greater number of our dwarfed patients over a sufficient period of time to obtain clinical results or to draw final conclusions.

As the years of adolescence approach, the patient should be carefully examined for signs of beginning sex development, and roentgenographic studies for observation of epiphyseal closure should be made. If there is considerable epiphyseal separation and the measurements are only slightly below the minimal normal, one can predict future and even sufficient statural growth to exclude that patient from being classified as a dwarfed adult. It is our observation that there is a deficiency not only in the pituitary growth factor but also in the sex factor as most of these youngsters are relatively retarded insofar as sex development and adolescence are concerned. They mature much later than the normally growing child. While they grow at a slow rate, they grow for several more years than the normal adolescent and consequently have a longer time in which to ultimately attain, whether treated or untreated, a reasonably normal height. It is more important to observe these patients for sex maturation and if they are delayed, to treat this rather than a moderate degree of statural underdevelopment.

Heredity is an undoubted factor in determination of ultimate growth. In the entire group there was only one instance in which true dwarfism was displayed in the family history (*case* 1, 4, 5 of group 2, brothers). The father, one paternal aunt and all 4 children in this family were pituitary dwarfs. However, the tendency to shortness of stature in the family was prevalent in the majority of all cases treated.

No attempt was made in any instance during the period of treatment to alter the diet of any of these patients. They all came from families who provided an adequate dietary regime. No vitamin therapy was at any time included. It is true, however, that the appetite of these youngsters under growth-stimulating treatment was increased instinctively without any urging on the part of the parents.

SUMMARY

Eighteen pituitary dwarfs with 4 exceptions, while treated with preparations of the pituitary growth factor, gained a greater increase in height than the normal increment for the time treated, while during periods of no treatment their gain was less than the normal increment.

In those cases in which pituitary dwarfism and infantilism are associated a preparation containing other factors of the anterior lobe than the growth factor might be more efficacious.

In early adolescence, sexual underdevelopment is more important than moderate statural deficiency and should be given preference in treatment. The state of epiphyseal closure as an indication of how much longer growth will remain possible is a factor in the decision as to the indication for treatment with the growth hormone.

The ultimate treatment of pituitary dwarfism has as yet not been attained. Further advances in the experimental field and better diagnostic ability will aid greatly.

Appreciation is expressed to Jean Schaefer for aid in the preparation of the manuscript and tables.

REFERENCES

1. Putnam, T. J., H. M. Teel and E. B. Benedict: *Am. J. Physiol.* 84: 157. 1928; *Arch. Surg.* 18: 1708. 1929.
2. Bugbee, E. P., A. E. Simond and H. M. Grimes: *Endocrinology* 15: 41. 1931.
3. Smith, P. E.: *Proc. Soc. Exper. Biol. & Med.* 30: 9. 1933.
4. Schaefer, R. L.: *Endocrinology* 20: 1. 1936.
5. Engelbach, W., and R. L. Schaefer: *Endocrinology* 18: 3. 1934.

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PITUITARY FUNCTION IN ADIPOSO-GENITAL DYSTROPHY (FRÖHLICH)[1]

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SINCE THE syndrome of dystrophia adioposogenitalis was first described by Fröhlich (1) in 1901, its pathogenesis was generally assumed to be inherent in some aberration of the pituitary gland. As early as 1909, objections to the pituitary origin of the disease were heard (2). Pathologic reports have demonstrated lesions of the hypophysis, of neighboring cephalic structures such as the infundibulum or infundibulo-hypothalamic region and indeed cases with no demonstrable organic disease.

In the light of more recent knowledge a wider approach to the pathogenesis of Fröhlich's syndrome becomes possible. A series of children displaying a typical picture of the disease were studied from the point of view of (*a*) fat metabolism hormone concentration of the blood; (*b*) carbohydrate metabolism as reflected not alone in the dextrose tolerance test but in their sensitivity to insulin, their hyperglycemic response to epinephrin and their response to the Staub-Traugott effect; (*c*) sensitivity to the induction of ketonemia on a high fat diet and (*d*) response to administration of the gonadotropic principle of pregnancy urine.

Fat Metabolism Hormone Concentration

In 1930, Burn and Sing (3) demonstrated that an alkaline extract of the anterior pituitary will influence the ketone excretion of rats on high fat diets. Anselmino and Hoffman (4) whose studies by report were substantiated by Boenheim and Heimann (5) and Bix (6), postulated the existence of a "fat metabolism" hormone of anterior pituitary origin. Zondek (7) has denied its existence and Thompson (7a) expressed the opinion that much of the work in connection with this hormone will need revaluation.

The rise of ketone bodies in the blood serum of male rats was determined after the injection of serum from 25 of our cases of Fröhlich's syndrome and compared with that produced by 22 normal children of the same age group. The difference between the total acetone value before and after injection was then expressed as percentage increase or decreased as compared to original ketone concentration. The determinations were made by the method of Benedict and Behre (8). If the ability of the human serum to increase the ketone concentration of the blood of the rat can be used as an index of concentration of circulating fat metabolism hormone, then the analytic data (fig. 1A) warrants the following conclusions. An average of 7% increase in the blood ketones was produced by our cases of Fröhlich's syndrome. Within the limits of error, no significant changes were produced by normals. This seems to indicate that in adiposogenital dystrophy there is a tendency for higher concentration of fat-metabolism hormone. From the data will be noted also that following an intensive

[1] This study was made possible by a Grant from the Child Neurology Research Fund of the Friedsam Foundation.

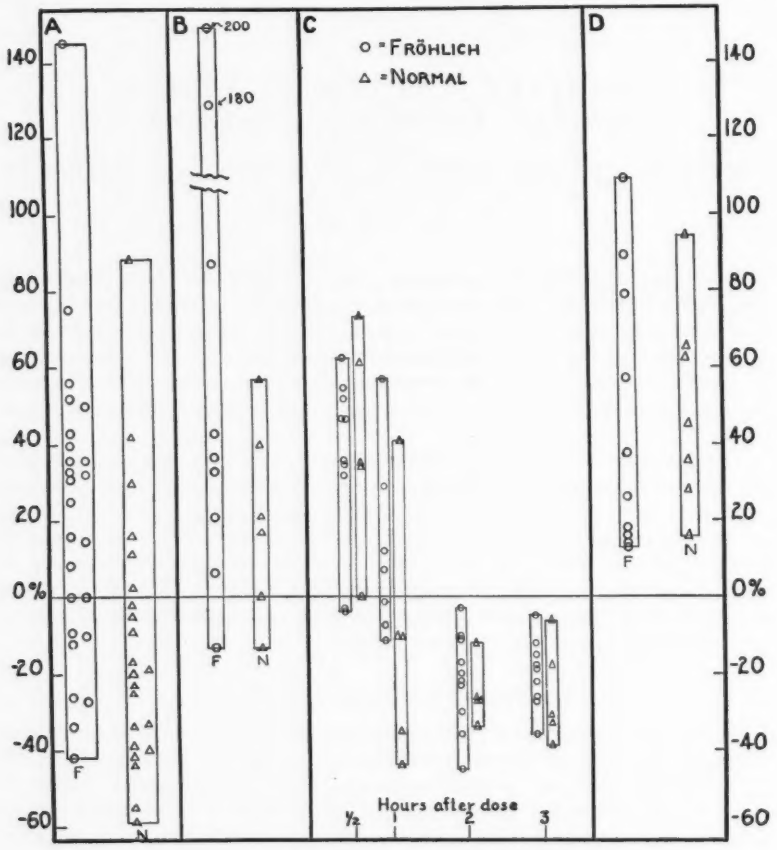

Fig. 1.

course of therapy with gonadotropic principle significant increases in the degree of ketonemia was produced.

Susceptibility to ketonemia following high fat test meal. The close relationship of the hypophysis to carbohydrate and fat metabolism would lead one to suppose that children with Fröhlich's syndrome would respond differently from normal children as regards their susceptibility or resistance to the induction of ketonemia. We therefore determined the blood ketone body levels in a series of 9 children with adiposogenital dystrophy and in 6 normals of the same age group after administration of a standard fat test meal (90 cc. of 40% cream). Three hours later oxalated blood was drawn for ketone body determination for comparison with ketone value, determined on fasting specimens. From figure 1B, it becomes apparent that the increase in blood ketones following a high fat test meal occurs in both Fröhlich's syndrome cases and in normals. The greatest percentage increase occurred in the former type of cases.

Carbohydrate Metabolism

Insulin sensitivity. The hypoglycemia following insulin administration can be

diminished by the administration of the diabetogenic principle of the anterior pituitary lobe of the hypophysis (9, 10). Hypophysectomized animals are markedly sensitive to the administration of insulin. It was therefore undertaken to utilize insulin sensitivity as an index of the hypophyseal function and a series of 10 cases of Fröhlich's syndrome along with a series of 5 normals were given a standard test dose of insulin, following which at intervals of ½ hour, 1 hour, 2 hours and 3 hours, blood sugar levels were determined. From figure 1C, it can be seen that there is no essential difference in the response of the abnormal from the normal case.

Blood sugar response to epinephrin. The hyperglycemic response to epinephrin gives some insight into pituitary function. In the hypophysectomized animal, the administration of epinephrin will not ameliorate the hypoglycemia which normally ensues (9). It was therefore undertaken to utilize the hyperglycemic response to

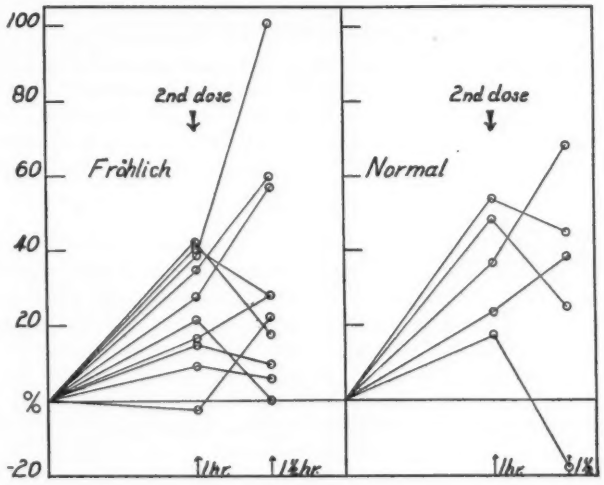

Fig. 2.

epinephrin as an index of hypophyseal function. Standard test doses of epinephrin to groups of 10 Fröhlich's syndrome cases and to 7 normals resulted in fluctuations of blood sugar which were remarkably alike in both groups studied (fig. 1D).

Staub-Traugott phenomenon. In 1921, it was determined (11, 12) that the administration of a second dose of glucose to a subject undergoing a glucose tolerance test at the time the blood sugar begins to drop, following the initial phase of hyperglycemia, results normally in little or no secondary rise in the blood sugar. It is the opinion in some quarters that the Staub-Traugott effect serves as a useful index of hypophyseal function (13) since the hypophysectomized animal may display a normal dextrose tolerance but will not yield the normal Staub-Traugott effects. The intact pituitary is therefore regarded as indispensable to the production of this phenomenon. Figure 2 represents the fluctuations in blood sugar resulting from the Staub-Traugott effect. No qualitative difference between 9 cases of Fröhlich's syndrome and 5 normals could be noted.

Gonadotropic Principle of Pituitary Gland

The significance of abnormal elaboration of gonadotropic principle by the

pituitary in relation to the hypogonadal state in Fröhlich's syndrome is so fundamental that it was undertaken to determine whether children displaying the Fröhlich habitus show aberrations in the concentration of the gonadotropic principle in their blood sera. In normal children, recognizable concentration of gonadotropic substance in the blood serum before puberty has been recorded (14, 15). The sera from our cases with adiposogenital dystrophy who had undergone no therapy with gonadotropic principle were assayed in accordance with the technics described by Freed (16) Evans (17) and Chen and Van Dyke (18). Preliminary determinations have indicated to us that by these technics no demonstrable gonadotropic principle is determinable in either the normal children or those with Fröhlich's syndrome. Further lines of investigation will be pursued to learn whether this lack of hormone is real or apparent.

Observation of Effect of Administration of Gonadotropic Principle in Cases of Adiposogenital Dystrophy

The results obtained with gonadotropic principle extracted from pregnancy urine (antiuitrin-S) in 6 of our cases of Fröhlich's syndrome resulted in definite genital development in 3 cases. No demonstrable effect on obesity was noted. The effect of such therapy on the concentration of fat metabolism hormone was determined in 3 cases and recorded under the general consideration of fat metabolism hormone.

SUMMARY

An investigation of the pituitary function in cases of adiposogenital dystrophy reveals the following. The rise in blood ketones in the rat produced by the injection of sera from cases of Fröhlich's syndrome reveals a tendency (not absolute) toward a greater increase in these ketones than were produced by the sera of normal children which seems to indicate a tendency toward greater concentration of ketogenic hormone. The degree of ketonemia following a high-fat test meal may be higher or decrease to the same degree as occurs in normals. Both normal children and those with Fröhlich's syndrome respond essentially in the same way to the administration of insulin, epinephrin and to the Staub-Traugott effect. Available technics to date have not in our hands shown demonstrable amounts of gonadotropic principle in the sera of either normal children or those with Fröhlich's syndrome. Administration of anterior pituitary-like gonadotropic hormone in 3 cases produced gonadal development. The obesity was entirely unaffected by such therapy.

REFERENCES

1. Fröhlich, A.: *Wien. Klin. Rundschau* 15: 883. 1901.
2. Erdheim, J., and E. Strumme: *Beitr. z. path. Anat. u. z. allg.* Path. vol. 46, 1909.
3. Burn, J. H., and H. W. Sing: *J. Physiol.* 119: 69. 1930.
4. Hoffman, F., and K. J. Anselmino: *Klin. Wchnschr.* 10: 2380. 1931.
5. Boenheim, F., and F. Heimann: *Ztschr. f. d. ges. exper. med.* 83: 637. 1932.
6. Bix, H.: *Wien. Med. Wchnschr.* 45: 686. 1932.
7. Zondek, B.: Diseases of the Endocrine Glands, Wm. Wood & Co. Baltimore, 1936.
7a. Thompson, D. L.: *A. Research Nerv. & Ment. Dis. Proc.* 17: 257. 1938.
8. Behre, J. A., and S. R. Benedict: *J. Biol. Chem.* 70: 487. 1926.
9. Corkrill, A. B., H. P. Marks and M. F. White: *J. Physiol.* 80: 193. 1933.
10. Smith, P. E., L. Dotti, H. H. Tyndale and E. Emgle: *Proc. Soc. Exper. Biol. & Med.* 34: 247. 1936.
11. Staub, H.: *Ztschr. f. klin. Med.* 91: 44. 1921.
12. Traugott, K.: *Klin. Wchnschr.* 1: 892. 1922.
13. Soskin, S.: *Am. J. Physiol.* 116: 148. 1936.
14. Freed, S. C.: *Proc. Soc. Exper. Biol. & Med.* 33: 35. 1935–1936.
15. Hess, I. H., R. H. Kunstadter and W. Saphir: *J.A.M.A.* 108: 358. 1937.
16. Freed, S. C.: *Proc. Soc. Exper. Biol. & Med.* 33: 309. 1935–1936.
17. Evans, H. M., M. E. Simpson and P. R. Austin: *J. Exper. Med.* 58: 561. 1933.
18. Chen. S., and H. B. Van Dyke: *Proc. Soc. Exper. Biol. & Med.* 35: 491. 1936–1937.

CLINICAL METHOD FOR ESTIMATING MONTHLY OUTPUT OF ESTRIN FROM SINGLE BIOASSAY ON POOLED NIGHT SPECIMENS OF URINE[1]

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THE PURPOSE of this communication is to report studies toward the development of a method for estimating the total intermenstrual output of urinary estrins by means of a single bioassay on pooled specimens of urine collected at night from 8 P.M. to 8 A.M. for one month.

MATERIALS AND METHODS

Urine specimens used in this study were obtained from psychotic patients at the Pennsylvania Hospital. Previous studies (1) have shown that the urinary excretion of estrin in psychotic patients is essentially the same as in non-psychotic patients. The accurate collection of specimens was assured by special nursing care. In each case the urine was collected in 12-hour periods, and all the night urine was added in a common vessel until the desired number of specimens had been collected. The day specimens were similarly pooled in another container. Both specimens were kept on ice and, by addition of small quantities of concentrated hydrochloric acid, were kept acid to litmus. No preservative was added. Bioassay of the estrin content was performed on aliquots of these by the technic previously reported by us (1) which is, essentially, a modification of the methods of Frank and Goldberger (2) and Smith and Smith (3).

Keeping properties of urinary estrins. Previous studies (1) have shown that free estrin increases rather than diminishes on standing. From subsequent assays, we have obtained estrin values in hydrolyzed specimens which did not deteriorate after standing for as long as 4 months. Thus, it seems certain that there is no significant deterioration when a month's urine is collected and pooled for assay.

Relation between day and night urine volume. The volume of urinary excretion under standard conditions has been studied by Campbell and Webster (4). They recorded the average volume output per hour during periods of rest, ordinary activity and measured exercise. Their day specimens were collected for a period of 14 hours, and night specimens for the remaining 10 hours. The ratio of day to night average hourly volumes was respectively 2.11, 2.26 and 2.11.

Obviously, the ratio between day and night urine volume may be altered by gross changes in fluid intake, by changes in external temperature and by certain diseases such as nephritis and diabetes. However, in those of our patients who had reasonably normal drinking habits, the ratio between day and night volumes over a period of a month was remarkably constant. The Day: Night ratio averages approximately 2: 1 in the cases listed in table 1.

[1] Aided by a grant from the John and Mary R. Markle Foundation to the Institute of the Pennsylvania Hospital.

TABLE 1

Case	Day volume	Night volume	Ratio, day:night
A	28 liters	14 liters	2.00
B	34 liters	16 liters	2.12
C	31 liters	16 liters	1.93
D[1]	13 liters	6 liters	2.16

[1] Specimen *D* was collected for a 10-day period only.

Estrin content of day and night urines. A preliminary study of the estrin content for full inter-menstrual periods in the cases of 5 female patients was undertaken. The average for the day urines was 54.2 mouse units of estrin per liter. The night urines yielded an average of 40.7 M.U. per liter. The test mice in these experiments were unselected adult female castrates. While there was considerable variation in the individual assays, the averages as noted above were considered sufficiently similar to warrant further studies.

In the second series, our test animals were selected as follows. About 100 young adult virgin female mice were examined by vaginal smear. Only those showing full vaginal cornification and having approximately the same weight were chosen. The rest were discarded. The selected mice were then all castrated on the same day and were used for assay exactly 7 days later, after which they were discarded. Using as the indication for the presence of one mouse unit full vaginal cornification in at least 3 out of 4 mice, we obtained in all instances remarkably clear-cut end-points. The results are noted in table 2.

TABLE 2

Specimen	No. days	No. liters	M.U./liter	M.U./specimen	Observed total M.U.	Calculated total M.U.
A Day	35	34.6	75	2595		
Night	35	16.2	75	1215	3810	3645
B Day	28	16.0	150	2400		
Night	28	10.2	150	1530	3930	4590
C Day	7	9.9	100	998		
Night	7	3.8	125	478	1476	1424
D Day	3	3.1	100	310		
Night	3	2.0	100	200	510	600

In the above table, one notes that the number of M.U. of estrin per liter is identical in day and night specimens from the same patient except in one instance in which the night urine contained 25 M.U. more per liter than the day urine. It would thus seem that the estrin content of the night specimens is a reliable guide to the estrin output for the full 24-hour period. The total number of M.U. of estrin for the combined day and night volumes have been calculated from the night urine alone, and are recorded in the last column of table 2. The formula Night M.U. $\times 3$ = total M.U. was used.

In specimen *A* the correlation between observed and calculated values is excellent. Specimen *B* is from a patient whose drinking habits were abnormal and the calculated total estrin output has an error of approximately $+17\%$. The periods of collection in specimens C and *D* are too short for an accurate comparison of volume, but are presented as data for the per-liter estrin content of day and night specimens.

It will be noted that the average estrin yields per liter are higher in these cases in which 7-day castrates were used than in the case of the unselected castrates. This is to be expected, since a freshly castrated mouse is a more delicate test animal.

DISCUSSION AND CONCLUSIONS

It has been shown that under normal conditions of fluid intake, that the relation of day to night urine volume is reasonably constant; that estrins do not deteriorate in the urine when kept on ice without preservative for longer than one month; that the use of selected fresh castrate mice materially increases the accuracy of estrin assay, and that, by our present methods of assay, the estrin content per liter of urine is essentially similar in both day and night specimens.

The total monthly output of estrin in the urine is assumed to be an index of ovarian function. An accurate estimate of this cannot be obtained from assay of a single 24-hour specimen because of the very large variations in estrin excretion at various times in the menstrual cycle. Assay of consecutive 72-hour specimens throughout the inter-menstrual period is too laborious and expensive for routine clinical use.

The work reported here indicates that the total monthly estrin output can be calculated from a single bioassay of an aliquot of the total night urine saved for 1 month. This brings the procedure within the means of the average patient, and permits the patient to engage in normal diurnal activity, since only the night urine need be collected.

REFERENCES

1. Lansbury, J., and J. Hughes: *Am. J. Psychiat.* 95: 1119. 1938.
2. Frank R. T., and M. A. Goldberger: J.A.M.A. 94: 1197. 1930.
3. Smith, G. V., and O. W. Smith: *Am. J. Physiol.* 112: 340. 1935.
4. Campbell, J. A., and T. A. Webster: *Biochem. J.* 15: 660. 1921.

The authors acknowledge with appreciation the technical assistance of Mrs. Barbra Dicke, and the cooperation of the nursing staff of the Pennsylvania Hospital.

EFFECTS OF LARGE DOSES OF PROTAMINE ZINC INSULIN IN NON-DIABETIC INDIVIDUALS[1]

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THE ACTION AND USE of protamine zinc insulin in diabetes have been the subject of numerous and thorough studies, but the effect of protamine zinc insulin on non-diabetic individuals on unrestricted diets has received practically no attention. Reese and VanderVeer (1) reported attempts to produce insulin shock for therapy of schizophrenia with protamine zinc insulin but treated their patients only briefly because they found it unsuitable to produce shock.

In an attempt to investigate several practical and theoretical questions regarding the effect of insulin in schizophrenia, 24 schizophrenic patients were subjected to treatment with protamine zinc insulin. No attempt was made to produce insulin shock in the manner of Sakel, indeed this state was avoided whenever possible, because it was found early in the work that patients treated with protamine zinc insulin were not often subject to shock even at high protamine zinc insulin dosage and with relatively low blood sugar levels. The patients were allowed up and about the ward at will. The resulting observations are more interesting as effects of protamine zinc insulin on non-diabetic individuals than as records of therapy of schizophrenia and will be presented from such a point of view.

METHOD

Schizophrenic patients who were inmates of Longview State Hospital were chosen more or less at random. The only requirements to be met were apparently safe physical health and permission from a responsible relative to give the treatment. Blood sugar tolerance curves following ingestion of 100 gm. of dextrose were determined in most instances before beginning treatment. The diet was the routine ward diet, supplemented by carbohydrates between meals when it seemed necessary. Early in the work initial doses were small. Patients were started on one dose of 15 U of protamine zinc insulin once daily, timorously increased at first by 10 U and then a second daily dose added. It was soon found that most individuals could readily tolerate larger amounts. Initial doses were then made 25 U twice daily at 12-hour intervals. These were increased step-wise every 4 to 10 days by increasing first the morning and then the afternoon dose by 25 U. In this way doses of 150 U twice daily were achieved in some individuals. The tolerance of individual patients varied greatly but most patients tolerated over 100 U daily as shown in table 1. The treatment was carried out over varying periods from 16 to 98 days until the patients had achieved a maximum of benefit or were deemed of no further interest for the study.

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[1] Some of the protamine zinc insulin used in this study was supplied gratis through the courtesy of the Eli Lilly Company and E. R. Squibb & Sons.

[2] Dr. E. A. Baber, Superintendent.

RESULTS

Sugar tolerance (alimentary, after 100 gm. of dextrose) curves (fig. 1) showed no significant change after the period of treatment with protamine zinc insulin. Whatever change that was evident can be accounted for by the improved physical state of the patients or simple individual variations. The recorded blood sugars were determined on ZnOH filtrates in which non-fermenting reducing substances were reduced to a minimum, so the initial levels were not really low for the method used.

TABLE 1

Case No.	Age and sex	Days of R_f	Max. and[1] min. bl. sugar	Max. daily dosage and No. of days maintained		No. of shocks[2]	Results in 1 yr.
			mg. %	U	*days*		
1	25 ♂	86	150; 13	150	6	2	C*; C after reg. insulin
2	31 ♂	98	131; 15	150	20	1	C*; B after reg. insulin
3	22 ♂	94	93; 11	200	23	7	A
4	25 ♀	83	83; 10	175	17	10	C*
5	24 ♀	83	93; 24	200	10	3	D*
6	40 ♀	8	19	50	5		Permission withdrawn
7	25 ♀	70	116; 17	200	10	4	C*; B after reg. insulin
8	28 ♀	66	111; 27	175	5	9	D*; D after reg. insulin
9	19 ♂	70	80; 24	250	15	1	C*; D after reg. insulin
10	26 ♂	62	75; 19	200	34	3	C*
11	32 ♂	62	61; 14	200	34	12	D*; D after metrazol
12	22 ♂	62	89; 11	200	34	4	C
13	32 ♀	45	132; 21	250	10	6	D
14	23 ♀	45	61; 17	250	10	3	D
15	31 ♂	39	61; 21	250	4	4	C
16	26 ♀	34	28	250	11	3	D
17	21 ♂	42	45; 31	300	15	0	A 2 yr. after
18	21 ♂	39	41; 15	250	8	0	C*; A after reg. insulin
19	30 ♀	36	124; 27	300	4	6	C*; C after reg. insulin
20	22 ♀	65		250	23	29	C*; A after reg. insulin
21	38 ♂	97		250	13	22	B*; B after reg. insulin, relapsed
22	36 ♀	16		200	2		C*; A after reg. insulin
23	46 ♀	85		100	9	8	D
24	25 ♀	27	250; 30	150	17		C*; A after reg. insulin

* Indicates that the patient received subsequent treatment of another sort.

[1] The maximum and minimum blood sugars are the maximum and minimum on the record throughout the whole period of treatment.

[2] Shock includes all states of insulin reaction from sweating and tremor to complete unconsciousness. Very rarely was this equal to the depth of coma regularly produced in the usual insulin therapy. The number of shocks indicated is the number that occurred throughout the period of treatment in any one patient.

The daily activity of the patients in relation to the blood sugar levels is of some interest. The patients were all allowed up and about the ward. Some would help with minor tasks or push floor polishers, according to their mental states. It was not uncommon for patients with blood sugars 50 mg. per 100 cc. or lower to be comfortably and normally active. At lower levels (below 40 mg. per 100 cc.) symptoms of insulin reaction were likely to occur. These when observed were treated immediately by oral administration of 6 to 8 oz. of sweetened orange juice. Not infrequently patients were not observed until in a semistupor. Even in such instances patients could be induced to drink.

Occasionally gavage or intravenous administration of dextrose had to be resorted to. This occurred most frequently on mornings when breakfast was delayed for blood

or electrocardiographic examinations. Blood sugar levels were most frequently below 40 mg./100 cc. in early morning hours. At other times the level of the blood sugar was much more variable and often given to huge fluctuations, particularly to high levels after food intake. The daily blood sugar curves of patients showed great individual differences in this respect (fig. 2, 3). In spite of high blood sugar levels no glycosuria was observed. A noteworthy point is that on the infrequent occasions when intravenous administration of dextrose had to be used, 10 to 20 cc. of 50% solution was adequate to bring the patients back to consciousness. This is certainly

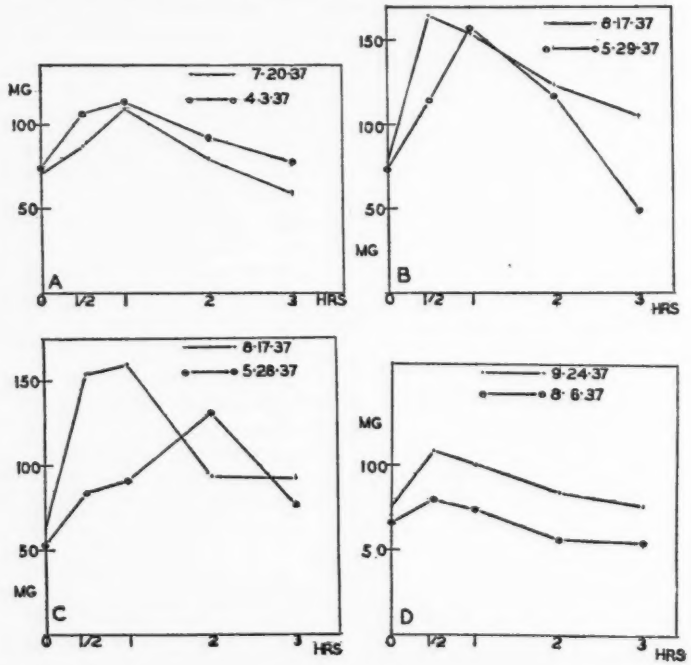

Fig. 1. A. *Case 1.* Sugar tolerance before (4-3-37) and 14 days after (7-20-37) protamine zinc insulin treatment. 100 gm. dextrose is the standard dose used. B. *Case 7.* Sugar tolerance before (5-29-37) and 7 days after (8-17-37) protamine zinc insulin treatment. C. *Case 8.* Sugar tolerance before (5-28-37) and 8 days after (8-17-37) protamine zinc insulin treatment. D. *Case 18.* Sugar tolerance before (8-6-37) and 7 days after (9-24-37) protamine zinc insulin treatment.

not the usual experience in regular insulin shock therapy. Also to be recorded is the fact that no clear cut epileptiform seizures occurred in protamine zinc insulin patients, although later the same patients often had such seizures while on regular insulin shock therapy.

One instance of serious reaction occurred, fortunately not attributable to the treatment. *Case 1* was given 75 U of regular insulin through an error during the period when he had been saturated at a level of 75 U of protamine zinc insulin twice daily. Within an hour the patient's behavior was found to be disturbed and quite unusual for him. An unfamiliar night attendant did not summon the physician for another hour. By that time the patient was fairly deeply unconscious. The blood sugar was down to 20 mg./100 cc. By dint of an all night vigil and frequent intra-

venous administration of dextrose the patient recovered and after a few days rest resumed the treatment with no apparent ill effect. This instance is interesting considering the case reported by Groen and Garrer (2) in which death followed adminis-

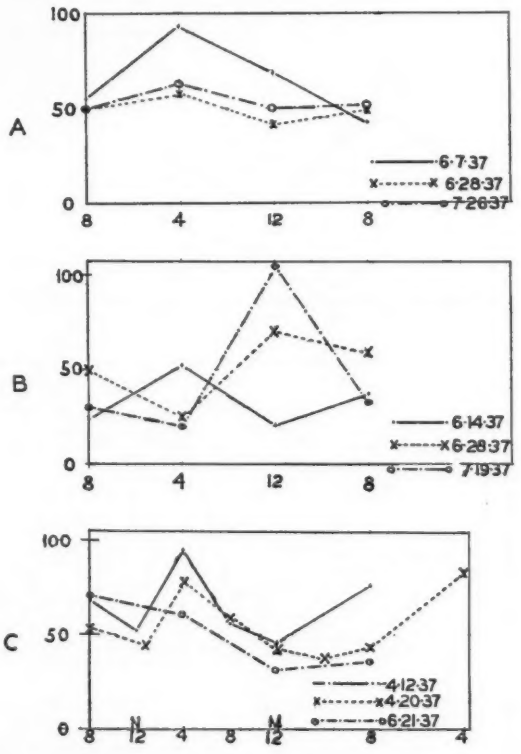

Fig. 2. A. *Case 5.* Blood sugars every 8 hr. during 24-hour periods.
6-7-37, 20th day of treatment, 50 U, A.M., 25 U P.M., protamine zinc insulin.
6-28-37, 41st day of treatment, 50 U A.M., 75 U P.M., protamine zinc insulin.
7-26-37, 69th day of treatment, 100 U A.M., 100 U P.M., protamine zinc insulin.
B. *Case 7* Blood sugars every 8 hr. during 24-hour periods.
6-14-37, 14th day of treatment, 50 U A.M., 25 U P.M., protamine zinc insulin.
6-28-37, 28th day of treatment, 50 U A.M., 75 U P.M., protamine zinc insulin.
7-19-37, 47th day of treatment, 75 U A.M., 100 U P.M., protamine zinc insulin.
C. *Case 1.* Blood sugars every 4 or 8 hr.
4-12-37, 7th day of treatment, 50 U A.M., 50 U P.M., protamine zinc insulin.
4-20-37, 15th day of treatment, 75 U A.M., 75 U P.M., protamine zinc insulin.
6-21-37, 73rd day of treatment, 25 U A.M., 25 U P.M., protamine zinc insulin.

tration of only 20 U of regular insulin in a protaminized diabetic in apparent insulin coma.

Practically all of the patients gained significant amounts of weight during the course of the treatment. In *case 3* this reached the remarkable amount of 45 lb. in 12 weeks (from 150 to 195 lb.). In most of these patients the excess weight gained was lost in the course of 6 months or a year.

Mental improvement occurred in many of the patients (table 2). The number

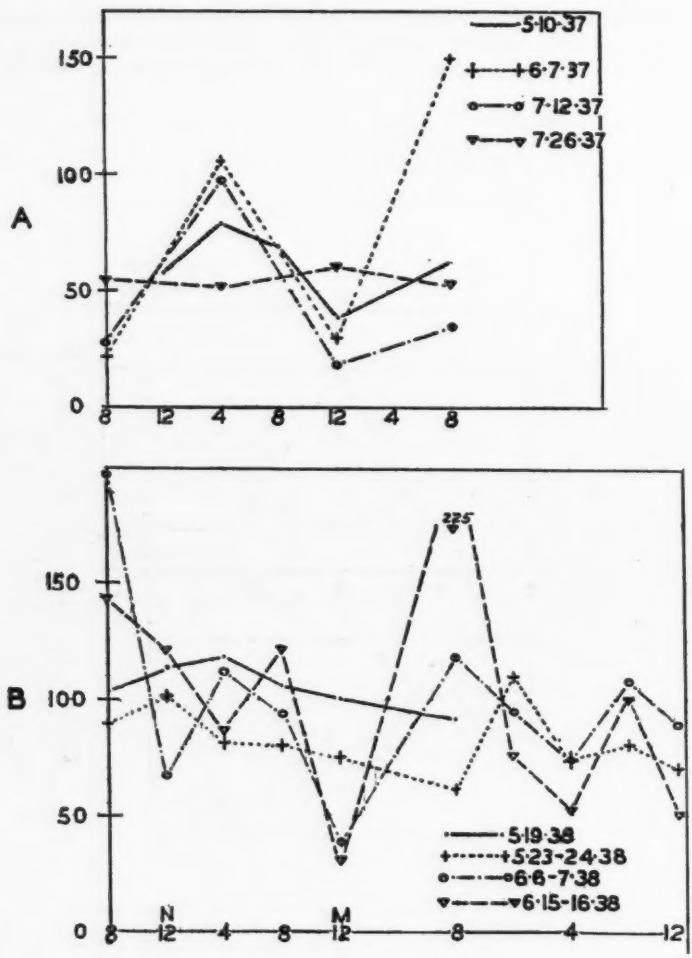

Fig. 3. A. *Case* 2. BLOOD SUGARS EVERY 4 OR 8 HR.
5-10-37, 16th day of treatment, 50 U A.M., 50 U P.M., protamine zinc insulin.
6- 7-37, 44th day of treatment, 75 U A.M., 50 U P.M., protamine zinc insulin.
7-12-37, 79th day of treatment, 75 U A.M., 75 U P.M., protamine zinc insulin.
7-26-37, 93rd day of treatment, 25 U A.M., 25 U P.M., protamine zinc insulin.
B. *Case* 24. BLOOD SUGARS EVERY 4 OR 8 HR., 40-HOUR PERIODS.
5-19-38, before treatment, no insulin.
5-23–24-38, 4th and 5th days of treatment, 25 U A.M., 25 U P.M., protamine zinc insulin.
6-6–7-38, 18th and 19th days of treatment, 75 U A.M., 75 U P.M., protamine zinc insulin.
6-15–16-38, 26th and 27th days of treatment, 75 U A.M., 75 U P.M., protamine zinc insulin.

treated is not great enough to consider this significant but in those patients who are rated *A* the quality of the remission was no less than could be achieved with regular insulin shock therapy. It is also true, however, that a number of patients who improved only moderately on protamine zinc insulin responded brilliantly after being placed on regular insulin shock treatment.

TABLE 2. SUMMARY OF RESULTS

	Duration 0–1 yr.	1–2 yr.	over 2 yr.
A—apparently complete recovery	2	0	0
B—recovery short of complete but allowing patient to resume normal activities	0	1	0
C—definite improvement less than B	1	5	7
D—no change after treatment	0	0	7

Electrocardiographic studies in a group of these patients showed changes equal to those seen under regular insulin treatments (3, 4) (fig. 4). These changes were only slowly reversible in some instances after the treatment was terminated.

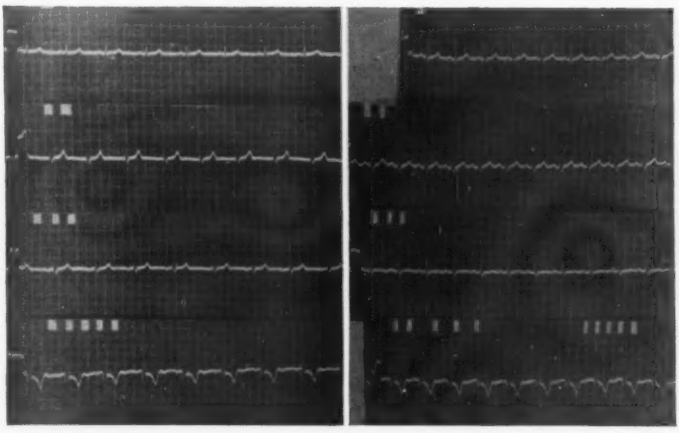

Fig. 4 *Left.* ELECTROCARDIOGRAMS OF *case* 2. BEFORE ANY TREATMENT 5-19-37. ESSENTIALLY NORMAL. *Right.* ON 85TH DAY OF TREATMENT 8-16-37, 8:00 A.M., PROTAMINE ZINC INSULIN, 100 U A.M., 75 U P.M. BLOOD SUGAR 37 MG./100 CC. MARKEDLY FLATTENED T_3. INCREASED VOLTAGE AND INVERSION (TO UPRIGHT) OF $P_{2,3}$. MARKED PHASIC VARIATION IN QRS VOLTAGE.

DISCUSSION

The outstanding fact reported is that non-diabetic individuals tolerated huge doses of protamine zinc insulin and most of the time were up and about and free of symptoms of hypoglycemia despite low blood sugar levels. Such levels were not necessarily of dangerous portent nor hard to control. Prolonged high dosage of insulin did not disrupt the patient's endogenous insulin mechanism or sugar tolerance. Noteworthy are inordinately high blood sugar levels even on high doses of protamine zinc insulin and relatively extreme fluctuations in the blood sugar levels. These findings may indicate inhibition of the patients' endogenous insulin secretion during the treatment, but this never persisted after cessation of the treatment. Such changes are in many respects similar to the observations of others in autogenous hyperinsulinism with islet cell tumors or hypertrophy (5). No complications such as are seen with insulin shock therapy and no deaths were encountered.

It is of some theoretical importance that hypoglycemia without shock was able to produce improvement in some individuals.

SUMMARY

The effect of protamine zinc insulin in large doses in non-diabetic individuals is described. The outstanding observations were: (*a*) relative lack of hypoglycemic symptoms even when blood sugar levels were low; (*b*) apparent safety of large doses of protamine zinc insulin (this does not necessarily apply to diabetics); (*c*) marked fluctuation of blood sugar levels during 24-hour periods; (*d*) marked transient gains in weight of most patients and (*e*) relative inefficiency in treatment of mental symptoms in schizophrenia.

REFERENCES

1. Reese, H. H., and A. VanderVeer: *Arch. Neurol. & Psych.* 39: 232. 1938.
2. Groen, J., and A. H. Garrer: *Neederland. Tijdschrift v. Geneeskunde* 83; 1844. 1939. (Abs. J.A.M.A. Vol. 113, 1939.)
3. Goldman, D.: *Arch. Int. Med.* To be published.
4. Bellet, S., H. Freed and W. W. Dyer: *Am. J. Med. Sci.* 198: 533. 1939.
5. Campbell, W. R., R. R. Graham and W. L. Robinson: *Am. J. Med. Sci.* 198: 445. 1939.

METABOLISM OF THE ESTROGENS

THE EFFECT OF LIVER AND UTERUS UPON ESTRONE, ESTRADIOL AND ESTRIOL[1,2,3]

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THIS STUDY indicates that liver tissue contains an enzyme system capable of destroying the biological activity of certain of the estrogens. Most tissues, and particularly the endometrium, are able to convert estrone into a more active estrogenic substance, which is possibly estradiol.

The immature rat uterus assay method of Lauson, Heller, Golden and Sevringhaus (1) was used to distinguish between estradiol, estrone and estriol. Twenty times more estrone is needed to elicit the same uterine weight response as that produced by estradiol. This can be appreciated by comparing the curve of response to doses of estradiol [fig. 1 (1)] with that of estrone [fig. 2 (1)], noting that the response to 0.3γ of estradiol is equal to that of 6.0γ of estrone.

In order to test the effect of rat and rabbit tissue slices upon estrogens, 5 cc. of a Ringer phosphate buffer at pH 7.4, containing 0.3γ/cc. of estrogen, was incubated with 250 mg. of tissue for one hour at 39° C. with continuous shaking. The incubated contents were then assayed biologically. Estradiol was found to be completely inactivated by liver slices and partially inactivated by kidney slices. Estradiol was not inactivated by incubation with heart, lung, spleen, uterine, or placental tissue (chart 1 and table 1).

The inactivation of estradiol following its incubation with liver slices could be explained by conversion to a less active form of estrogen, conjugation, or partial or complete destruction of the estradiol.

The probability of a conversion to the two less active estrogens, estrone and estriol, is ruled out by the completeness of the inactivation. Negative responses were obtained even when the assay dose levels were raised to 3 and 4 times the original amounts. Thus, had estradiol been converted into estrone or estriol, one would expect positive responses at these dose levels.

The possibility that the inactivation of estradiol, estrone and estriol by liver and kidney slices is due to the formation of an inactive conjugated form cannot be entirely ruled out. However, applying hydrolysis methods which usually lead to the liberation of these estrogens from their conjugated forms in urine did not cause such a release from estrogens inactivated by incubation with liver and kidney slices. A second factor strongly suggesting the unlikelihood of inactivation by conjugation is

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[1] Presented in part before the 51st Annual Meeting of the American Physiological Society, Toronto, Canada, April 27, 1939.

[2] This research was supported in part by a special grant from the Wisconsin Alumni Research Foundation and by a Research Fellowship provided by the Schering Corporation, both administered by Dr. E. L. Sevringhaus.

[3] We are indebted to Dr. G. Stragnell of the Schering Corporation for the supply of estradiol and estrone, and to Dr. Oliver Kamm of Parke, Davis & Co. for the estriol.

that the better known conjugated estrogens are nearly all active estrogenically. Two of the conjugated estradiols, estradiol benzoate and estradiol glucoside, were found to be active at the 0.3γ level. A third factor favoring the unlikelihood of conjugation is that the very tissues that might be expected to conjugate estradiol cause hydrolysis of both estradiol benzoate and estradiol glucoside and a consequent increase in potency. From table 2 it can be seen that those tissues which are most active in inactivating estradiol cause the least increase in potency of estradiol benzoate and estradiol glucoside. This seems reasonable since one can expect that liver and kidney would destroy the free estrogen as it is liberated from its ester form.

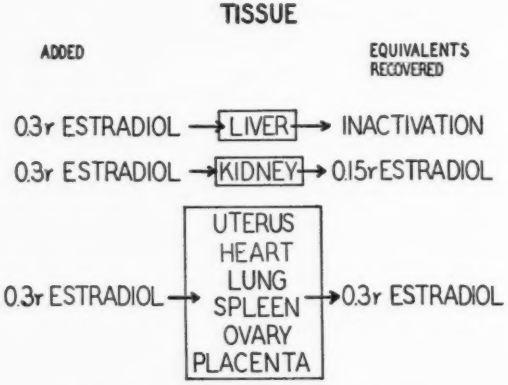

CHART 1. EFFECT OF TISSUE SLICES ON ESTRADIOL

Therefore, the idea of enzymatic destruction suggested itself. To test the proposal that the disappearance of estradiol from liver and kidney preparations following incubation was due to enzymatic destruction, various enzyme system inhibitors were introduced into the incubation procedure. Both non-specific and specific enzyme system inhibitors were used. Since many enzyme systems possess a protein structure, they are affected by the different protein reagents, and such an inhibition is considered to be non-specific, because all enzyme reactions under the influence of these reagents will be inhibited. There are also a large number of substances which appear to inhibit selectively a given enzyme reaction and these are considered to be specific. Most enzyme systems are destroyed by heating. Accordingly, when the liver slices were boiled before incubation with estradiol, the inactivating system was completely inhibited and all the added estradiol was recovered. Autolysis is known to destroy various enzymatic reactions of tissues, and this was true of the estradiol-inactivating system in liver which was inhibited by autolyzing the liver before adding the estradiol.

NaCN has no effect upon dehydrogenases, but does inhibit oxygenases strongly. An exception to the above is the NaCN inhibition of xanthine dehydrogenase. Uricase is also inhibited by NaCN. The addition of NaCN to the liver-estradiol incubation system caused complete inhibition of the inactivating system. This suggests that one of the above enzyme systems plays a rôle in inactivating estradiol, and the most likely of these is an oxygenase system.

Other enzyme system poisons caused no inhibition of the inactivating process in liver slices, and the destruction of estradiol continued as usual. These tissue poisons

included Na pyrophosphate, iodoacetic, malonic and maleic acids. Na pyrophosphate is known to inhibit iron catalyzed reactions and specifically inhibits succinic dehydrogenase. Iodoacetic acid inhibits reactions requiring free SH groups by combining with these groups. It inhibits the action of the lactic and alpha glyceric phosphoric acid dehydrogenase in muscle, aldehyde mutase, alcohol and alpha glyceric

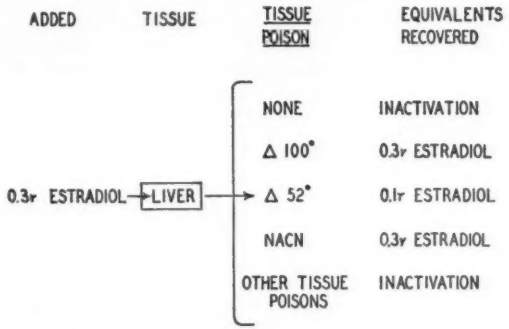

CHART 2. EFFECT OF ENZYME INHIBITORS UPON THE ESTRADIOL INACTIVATING SYSTEM OF LIVER

phosphoric acid dehydrogenase in yeast, and triose phosphate dehydrogenase. Malonic acid inhibits succinic, malic, lactic, fatty acid and alcohol oxidations and maleic acid inhibits glyoxalase and succinic dehydrogenase, Cohen (2). Thus the inability of these tissue poisons to affect the estradiol-inactivating system in liver would rule out the specific enzyme systems which these poisons inhibit. This leaves the likelihood that the inactivating mechanism found in liver is not only enzymatic but quite possibly oxidative in character.

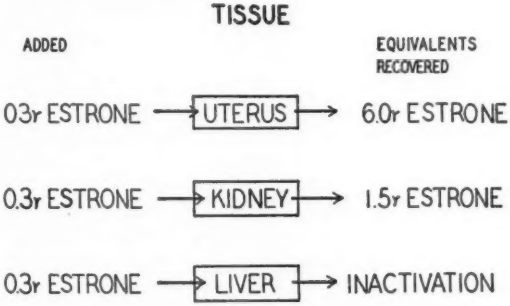

CHART 3. EFFECT OF TISSUE SLICES UPON ESTRONE

The fate of estrone was also studied when incubated with tissues under the same conditions and in the same amounts as estradiol. Liver slices inactivated estrone as before, but incubation with uterine tissue not only did not decrease its estrogenic activity but actually increased it. Thus, 0.3γ of estrone, after incubation with uterine tissue, elicited an assay response ordinarily given by 6.0γ of estrone. This is an increase of 2000%. All other tissues tested, except liver, increased the estrogenic activity of estrone, but none as much as uterine tissue. This includes spleen, kidney, ovary and heart (chart 3 and table 4).

Since uterine tissue had no effect whatever upon estradiol it is apparent that uterine tissue is capable of differentiating between the two estrogens. Assuming the action of uterine tissue in increasing the activity of estrone to be enzymatic, the various enzyme or tissue poisons were added to the system before incubation. None of the tissue poisons, however, inhibited the mechanism and estrone increased in activity as before. Only one exception to this was found, namely, boiling the uterine tissue. In this case the exact amount of estrone added was subsequently recovered (chart 4 and table 5).

Obviously, 0.3γ of estrone cannot spontaneously reproduce itself and become 6.0γ, even though it gives the effect of 6.0γ of estrone, so another explanation of its fate must be sought. The simplest is that the uterus converts estrone to another estrogen of greater biological activity. A hint that this might be estradiol was de-

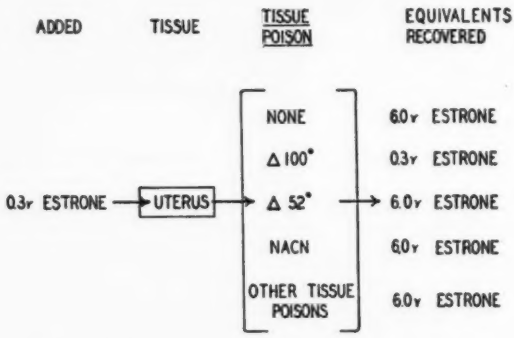

CHART 4. EFFECT OF ENZYME INHIBITORS UPON THE ABILITY OF THE UTERUS TO INCREASE THE POTENCY OF ESTRONE

rived from the observation that rat kidney (table 6), which only slightly inactivates estradiol, greatly increased the activity of estrone. Pregnant rat kidney, which partially inactivates estradiol, only slightly increased the activity of estrone, and rabbit kidney, which completely inactivates estradiol also completely inactivates estrone. In fact, whether estrone or estradiol were originally added to kidney made little difference in the assay response following incubation. The same observation was made about the incubation of estrone and estradiol with liver and with uterus (chart 5). That is, it made no difference which estrogen was added, the biological response was always approximately that obtained with estradiol alone. (Compare the 0.3γ figures for uterine tissue in table 1 with those in table 4.) As further evidence that the uterus possibly converts estrone to estradiol, it is well to note that the increase in potency is of the magnitude of 20 times that of 0.3γ of estrone, or 6.0γ. This is exactly the magnitude of increase that could be expected if estrone were converted molecule for molecule into estradiol since estradiol is 20 times as potent as estrone. It may be significant that upon occasions the increase was less than 20 times but was not more than 20. If the estrone which was increased in potency by incubation with uterine tissue was then added to liver tissue the latter inactivated it just as it does normally with estradiol. If one assumes that estrone is converted into estradiol, then, since estrone is a ketone and estradiol is an alcohol, the reaction would be one of reduction.

The concept that estrone may be converted into estradiol biologically is not entirely without precedent. Wettstein (3) has found that estrone is markedly changed chemically following incubation with a yeast substrate. The end-product of the incubation was tentatively identified as alpha estradiol. Smith, Smith and Schiller (4) have noted a biological increase in activity of urinary estrone following treatment with powdered zinc or following bacterial or enzymatic action during storage. They suggest that the increased activity is due to "conversion of estrone to a reduced form of greater estrogenic activity." They observed that zinc hydrolysis does not affect the activity of estriol or estradiol.

Thus there may well be two separate tissue actions upon estrogens in the animal body. One, in the liver, which may be considered to be brought about by an oxidative enzyme system which destroys estradiol; and a second in the uterus which does not

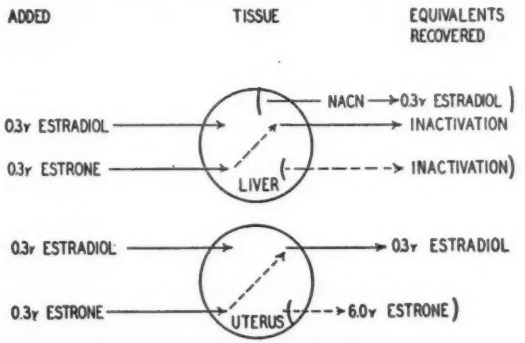

CHART 5. ILLUSTRATING THE SIMILARITY OF END PRODUCTS WHEN EITHER ESTRONE OR ESTRADIOL ARE ADDED TO UTERUS OR WHEN EITHER ARE ADDED TO LIVER. THE DOTTED DIAGONAL ARROW INDICATES THE AMOUNT OF ESTRONE RECOVERED WHEN EXPRESSED AS ESTRADIOL. END PRODUCTS ARE IDENTICAL FOR ESTRONE AND ESTRADIOL IN CYANIDE POISONED LIVER. A COMPARISON OF THE EFFECTS ON ESTRONE OR ESTRADIOL OF CYANIDE POISONED LIVER WITH NORMAL UTERUS SHOWS THE SAME END RESULTS.

act upon the alcohol, estradiol, but which presumably acts by enzymatic reduction to increase the activity of estrone.

Since most of the tissues tested tended to increase the potency of estrone, it was further assumed that estrone may be reduced to estradiol by the liver as well as by the other tissues and that the liver later oxidized the estradiol, causing it to disappear. If estrone is reduced to estradiol by liver slice before it is destroyed, then by poisoning the oxidative enzyme system with cyanide one could expect that estrone would increase in potency, because, after being converted to estradiol it could not be destroyed. To test this assumption the estradiol-destroying enzyme systems of the liver slices were poisoned with M/100 NaCN before adding estrone. As before, 0.3γ of estrone was added to the cyanide poisoned liver and incubated. Upon assaying, the estrone was found not to be decreased in amount, as usually occurs with normal liver but was increased in amount. Now the 0.3γ of added estrone, upon assay, gave the reaction of 6.0γ of estrone, or expressed in terms of estradiol, it gave the same reaction as 0.3γ of estradiol normally does (table 5). Thus cyanide poisoned liver and normal uterine tissue act alike upon estrone in that they both increase its potency as illustrated in chart 5. Cyanide poisoned kidney also greatly increases estrone potency (table 6).

Chart 6 illustrates the possible mechanism the two enzyme systems in liver have upon the metabolism of estrone with two steps in the inactivating process.

Since all biological assay methods are performed with intact animals it seems entirely conceivable that estrone itself may be an inert substance and only manifests a biological action when converted to estradiol. A very similar suggestion was made earlier (1) when it was found that while the estrone and estradiol uterine weight assay curves are quantitatively different, they are qualitatively alike. This, plus the greater variation in response of the estrone series, suggested that while estradiol may be utilized directly, estrone may be wholly or partly converted into another form in the animal body.

It seemed of interest to test the effect of tissues upon the metabolism of estriol,

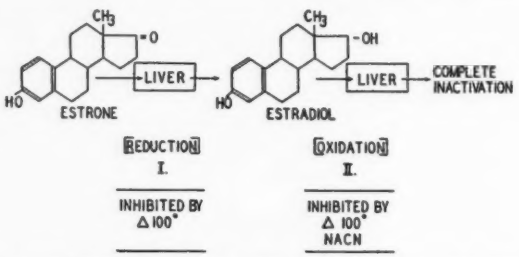

CHART 6. POSSIBLE MECHANISM OF THE LIVER IN INACTIVATING ESTRONE

a third but somewhat different naturally-occurring estrogen. Estriol differs from estradiol and estrone in the following ways. It is found in urine, but to date has not been isolated from ovaries. Increasing increments of dosage do not cause increasing increments of uterine weight beyond 60 mg. in immature rats and uterine ballooning does not occur (1). Estriol is more effective when administered orally than either of the two other estrogens.

Estriol was tested in the same dosages, with the same tissues and using the same procedures as were used to determine the nature of the metabolism of estrone and estradiol. Estriol was neither increased nor decreased in potency by rat uterus, spleen, lung or rabbit kidney. It was slightly decreased in potency by rat liver and kidney and by rabbit liver as shown in table 7. Ballooning and uterine responses over 60 mg. did not occur in any instance following incubation with any of the tissues used. This was true even when the assay dosages were increased several times. This would seem to rule out the possibility that estriol was being converted into either estrone or estradiol.

Therefore the metabolism of estriol differs from estrone in two important respects. (*a*), It is not increased in potency or converted to a more potent form by tissues, as estrone is. (*b*), It is not as completely destroyed by liver as estrone is. The metabolism of estriol resembles that of estradiol in that, with the exception of liver and kidney, no tissues act upon it. Estriol differs from estradiol in being but slightly inactivated by liver, whereas estradiol is markedly inactivated by liver tissue.

These differences between estriol and the other two estrogens could conceivably explain why estriol is more effective than estrone and estradiol when administered by the oral route. In order to reach the general circulation, following oral administration, the estrogens must pass through the liver via the portal circulation. It would seem reasonable, in light of the above findings, to expect that during the course of passage through the liver estrone and estradiol would be easily inactivated. Estriol

being more resistant to the estrogen inactivating properties of the liver would tend to pass through the liver and reach the general circulation in greater concentrations. This greater concentration of estriol might then account for the greater effect upon the end-organs usually found after oral administration. The experiments of Golden and Sevringhaus (5) tend to support this concept. They implanted ovaries into the portal circulation of castrated rats and found no peripheral evidence of any circulating hormone, whereas in thoracic implants normal estrogenic function continued.

It is obvious that many of the above results run counter to some of the current thought regarding the metabolism of estrogenic substances. This is particularly true of the experiments reported by Pincus and Zahl (6) and Smith and Smith (7). On the other hand, some of these findings confirm such reports about the action of the liver upon some of the estrogens as those by Zondek (8), Israel, Meranze and Johnston (9), Golden and Sevringhaus (5), Talbot (10), and Biskind and Mark (11). However, when making comparisons it must be kept in mind that the technics and approach used in this study differ materially from those used by other workers. These others made use of perfusion experiments, experiments with intact animals and the testing and identification of metabolized excretory products. Even the biological assay method herein used is not comparable to most methods, particularly not to the present unreliable colorimetric methods available for assaying small quantities of estrogenic substances. It might also be pertinent to point out that all chemical or other concentration methods were rigidly avoided, thus eliminating the hazard of loss or change of the estrogens.

SUMMARY

In vitro studies on the effect of tissue slices and tissue mince upon estradiol, estrone and estriol as judged by their biological activity are reported. Estradiol is inactivated by liver and kidney and not by the other tissues studied. This inactivation of estradiol is, in all probability, not due to conjugation or conversion to a less active form, but due to enzymatic destruction of an oxidative nature. This is indicated by the relative inactivity of the treated estradiol, the inability of hydrolysis procedures to increase activity and the effect of tissue poisons upon inhibiting the estradiol-destroying enzyme system.

Estrone is increased 20 times in potency by incubation with minced uterine tissue. Spleen, lung, heart and kidney tissues also caused estrone to be converted to a more active substance. The suggestion is made that this conversion is accomplished by enzymatic reduction of estrone to estradiol.

Liver tissue, which has been found normally to destroy estrone, when poisoned with NaCN causes an increase in estrone potency. Since NaCN also inhibits the estradiol-destroying system in liver it is suggested that liver contains both an estrone-reducing enzyme system which converts estrone to estradiol, and an estradiol-destroying system which inactivates the estradiol so formed. All respiring tissues studied caused the hydrolysis of estradiol glucoside and estradiol benzoate. An explanation is offered for the better results obtained with orally administered estriol than with orally administered estradiol and estrone, since estriol is only mildly affected by liver tissue in contrast to the great destruction of estrone and estradiol.

APPENDIX

TECHNICAL PROCEDURES

Each flask to be incubated with any given tissue contained, besides 250 mg. of tissue, enough of an estrogen solution and buffer to total 5 cc. of fluid. Each cc. of this fluid repre-

TABLE 1. ASSAY OF ESTRADIOL

Tissue	Theoretical amount of estradiol injected, γ	Uterine response, ave. weight in mg.			Number of assay rats		
		Controls	Incubation with rat tissues	Incubation with rabbit tissues	Controls	Rat tissues	Rabbit tissues
None	None	19.7			85		
None	0.3	89.3			25		
	0.9	90.0					
Liver	0.3		21.0	23.1		34	12
	0.9		21.8	42.3		6	8
	1.8		—	71.0		—	2
Kidney	0.3		72.1	26.9		30	9
	0.9		110.0	45.5		3	8
Uterus	0.3		84.1	81.5		15	11
Spleen	0.3		85.7	96.5		4	2
Heart	0.3		87.0	81.0		2	2
Lung	0.3		81.8	—		6	—
Placenta	0.3		—	85.0		—	2

TABLE 2. ASSAY OF CONJUGATED ESTROGENS

Tissue	Theoretical amount of estrogen injected, γ	Uterine response, wt. mg.	
		Estradiol benzoate	Estradiol glucoside
None	0.3	42	
	0.6	54	
	0.9	77	
None (hydrolyzed)	0.3	72	
Liver	0.3	54	29
Kidney	0.3	97	51
Uterus	0.3	88	—
Spleen	0.3	97	86

TABLE 3. EFFECT OF TISSUE POISONS ON THE INACTIVATION OF ESTRADIOL

Tissue slice	Tissue poison	Theoretical amount inj., γ	Uterine response ave. wt., mg.	No. of assay rats
None	None	None	19.7	85
None	None	0.3	89.3	25
None	NaCN	0.3	83.0	2
Liver	None	0.3	21.1	46
Liver	NaCN	0.3	89.0	7
Liver	NaCN	0.3	60.0[1]	8
Liver	Autolysed	0.3	100.0	3
Liver	Heat, 100°	0.3	84.0	5
Liver	Heat, 52°	0.3	49.0	2
Liver	Na pyrophosphate	0.3	16.0	2
Liver	Iodoacetic acid	0.3	24.0	2
Liver	Malonic acid	0.3	17.0	2
Liver	Maleic acid	0.3	15.0	2
Kidney	NaCN	0.3	99.0	3
Kidney	Heat, 100°	0.3		

[1] This liver tissue was incompletely killed at the time the NaCN was removed.

TABLE 4. ASSAY OF ESTRONE

Tissue	Theoretical amount of estradiol injected, γ	Uterine response, ave. weight in mg.			Number of assay rats		
		Controls	Incubation with rat tissues	Incubation with rabbit tissue	Controls	Rat tissues	Rabbit tissues
None	None	19.7			85		
None	0.3	19.7			85		
	1.0	38.5			34		
	2.0	55.6			28		
Liver	0.3		20	20		1	7
	0.9		25	22		6	4
	1.8		24	32		3	2
Kidney	0.3		48	24		6	5
	0.9		85	41		8	4
	1.8		110	59		1	2
Uterus	0.3		77[1]	78		6	20
	0.6		96[1]	—		2	—
	0.9		105[1]	100		3	11
Spleen	0.3		33	30		7	1
	0.9		61	56		2	2
Heart	0.3		24	—		2	—
	0.9		51	45		2	2
Ovary	None		—	21		—	1
	None		—	19		—	1
Ovary	0.3		—	53		—	1
	0.9		—	102		—	1

[1] Pregnant rat uteri were used here since non-pregnant figures are distinctly lower.

TABLE 5. EFFECT OF TISSUE POISONS ON THE INACTIVATION OF ESTRONE

Tissue slice	Tissue poison	Theoretical amount inj., γ	Uterine response, ave. wt., mg.	No. of assay rats
None	None	None	19.7	85
None	None	0.3	19.7[1]	85[1]
		1.0	38.5	34
Uterus	None	0.3	78.0	26
		0.9	101.0	14
Uterus	NaCN	0.3	80.0	5
Uterus	Heat, 100°	0.3	16.0	1
		0.9	32.0	1
Uterus	Heat, 52°	0.3	70.0	1
		0.9	98.0	1
Uterus	Na pyrophosphate	0.3	100.0	1
		0.9	89.0	1
Uterus	Iodoacetic acid	0.3	80.0	2
Uterus	Malonic acid	0.3	74.0	1
		0.9	98.0	1
Uterus	Maleic acid	0.3	90.0	1
		0.9	98.0	1
Liver	None	0.3	20.0	8
		0.9	24.0	10
Liver	NaCN	0.3	67.0	4
		0.9	100.0	3

[1] Control figures taken since the response here is below the minimal.

sented 0.3γ of estrogen. Following the incubation for one hour at 39°C. the solutions were kept at 0 to 5°C. Adsorption of the estrogen onto the tissue slices was eliminated as a source of error by finely macerating the slices and injecting them into the assay rats along with the remaining incubated solution. The assay dosage was planned to contain a total of 0.3γ,

TABLE 6. COMPARISON OF EFFECT OF KIDNEY TISSUE FROM SEVERAL SOURCES UPON THE METABOLISM OF ESTRONE AND ESTRADIOL

Estrogen	Theoretical amount of estrogen injected, γ	Rat	Pregnant rat	Rabbit	Pregnant rabbit	Estrogen injected controls
Estradiol	0.3	73.1	65.6	27	26.6	89.3
Estrone	0.3	56.0	40.0	—	23.6	19.7
Estradiol	0.9	—	111.0	48	38.0	>90.0
Estrone	0.9	81.0	87.0	47	35.0	38.5[1]
Estradiol+NaCN	0.3				100.0	
Estrone+NaCN	0.9				100.0	

[1] Control dosage was 1.0 instead of 0.9.

Uninjected control uteri average 19.7 mg.

Notice the large differences in uterine weight between the estrone and estradiol injected controls, and the small differences between the two following incubation with the various kidney tissues.

TABLE 7. ASSAY OF ESTRIOL

Tissue	Theoretical amount of estriol injected, γ	Uterine response ave. weight in mg.			Number of assay rats		
		Controls	Incubation with rat tissues	Incubation with rabbit tissues	Controls	Rat tissues	Rabbit tissues
None	None	19.7			85		
None	0.3	35.0			10		
	0.5	47.0			3		
	1.0	53.0			4		
Liver	0.3		27.2	28.1		16	10
	0.6		33.3			11	
	0.9		49.2	41.0		5	8
Kidney	0.3		27.5	39.5		24	9
	0.9			56.6			5
Uterus	0.3		41.0	41.0		5	8
	0.9			54.0			3
Spleen	0.3		37.2			4	
Lung	0.3		41.1			4	

0.9γ or 1.8γ of the added estrogen. Calculations were made on the assumption that no estrogen had been destroyed. Therefore destruction was demonstrated by the diminution of uterine response in the assay rat from that expected for the amount of estrogen added. All these responses after experimental procedures can be compared with the uterine responses of the injected controls in tables 1 and 4 [or with the assay curves fig. 1 and 2 in the previous paper (1)]. The uninjected controls serve to indicate at what uterine weight (16 to 24 mg.) too little estrogen is present to elicit a positive response. A total of 23 donor rats and rabbits were used to obtain tissues for 23 separate incubations. For almost every incubation a series of positive and negative controls were run under the same conditions as the experimental incubations. Thus, to test the effect of NaCN upon the ability of liver to destroy estrone the following controls were run: *a*), uninjected test rats to rule out colony variation, *b*), unincubated estrone injected into test rats to check on the response of the rats to the dosage of estrone used, *c*), both estrone and estradiol were incubated with normal liver to check the ability of the liver to destroy these estrogens, *d*), estrone was incubated with uterine

minced tissue in each case in order to establish that estrone could definitely be increased in potency and *e*), NaCN was added to estradiol before incubation with liver to make certain that the inactivating enzyme system could be inhibited. Then when it was found that NaCN inhibited liver caused a great increase in the potency of the added estrone, it was assumed that the estrone had been converted to estradiol and the estradiol in turn was not being inactivated, due to the cyanide. Only one to three rats were used to assay the contents of each flask. This was felt justifiable in view of the many check runs, the large number of individual experiments run (266 separate flasks used), the reliability of the end-point,[4] the fact that we were dealing with large differences and the fact that later experiments checked earlier data. (For example, all the data showing that tissue poisons do not inhibit uterine tissue conversion of estrone to a more potent estrogen tend to confirm the original data that uterus does cause an increase in estrone potency.)

Table 1 shows that rat and rabbit liver and kidney tissues are not equal in their ability to inactivate estradiol, so they have been listed separately. It will be noted that at the 0.3γ level all the estradiol has disappeared from the liver incubated solution in both rat and rabbit. At the 0.9γ level in the rat it is also absent despite the 3-fold increase in assay dose. This rules out a possible conversion to the less potent estrone, since, at this level [fig. 2 (1)] estrone elicits a positive uterine response. The positive responses of estradiol at the 0.9 and 1.8γ levels following incubation with rabbit liver is probably due to incomplete destruction of the estradiol and not conversion to the lower potency estriol since definite ballooning was present at the 1.8γ level.

Each combination of estrone, estriol and estradiol with liver or kidney was treated by both acid (HCl) and alkali (NaOH) hydrolysis. Both weakly acid and basic solutions and strongly acid and basic solutions were used. The pH ranged from 1 to 14. The solutions were autoclaved at 115°C. and 15 lb. pressure for 1 to 2 hours. Using this procedure no loss in potency was discernible when estradiol controls were similarly treated, even when boiled tissue was added. The alkali hydrolysis of untreated estradiol glucoside, which served as a positive control, was effective in liberating the free form of the estradiol, as judged by its efficiency in causing increased uterine weight in the test rats. But, no form of hydrolysis caused any increase in potency of estrone, estriol or estradiol that had previously been inactivated by incubation with kidney or liver slices.

Sodium cyanide in M/100 and M/50 concentrations was found to completely inhibit the inactivating properties of liver and kidney tissues upon estradiol as shown in table 3. The NaCN was rendered non-toxic by boiling it off before injecting solutions into the test rats. All other tissue poisons were used in concentration of M/100. The tissue poisons were added to the tissue slices at the same time the estrogen was added. The heated liver was heated to 52° or 100° before addition to the estrogen-buffer solution. The autolyzed liver was permitted to stand in the warm room at 38° for 4 days before being added to the solution. It had been determined previously that the enzyme system capable of inactivating the estrogens was not materially altered by standing at room temperature 1 to 2 hours before incubation.

As shown in table 4, estrone behaved similarly to estradiol only insofar as it was inactivated by liver. All other tissues caused an increase in potency of the estrone. The most efficient tissue in bringing about this increase was the uterus. In this case a uterine mince had to be used because slicing was found to be impractical. Pregnant rat uteri were more effective than the non-pregnant rat uteri in causing an increased potency because they contained sufficiently more endometrial tissue to bring about complete conversion. Endometrium alone was considerably more effective per unit weight than whole uterus or myometrium. Ovarian tissue slices also caused increased potency of estrone. Assaying ovarian and uterine tissue without the addition of estrogens showed that they contained insufficient amounts of estrogens to cause a response in the assay rats and not enough to augment the effect of estriol upon which uterine tissue had no effect.

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[4] C. Eisenhart has kindly analyzed the standardization data and reports part of the findings in "The Interpretation of certain Regression Methods and their use in Biological & Industrial Research," *Annals of Mathematical Statistics* 10: 162. 1939.

Both liver and kidney are able to increase the potency of estrone when their inactivating systems are prevented by NaCN from inactivating the higher potency estrogen formed. In fact, the detectable increase in estrone potency incubated with kidney is in direct proportion to the kidney's ability to destroy estradiol as seen from table 6.

The figures in table 7 are of interest because of the lack of change in the potency of estriol brought about by the various tissues. At the same time it was felt that the lower figures in the case of the liver, while they are not strikingly different from the standard, are not due to experimental error. The uterine weights of assay rats following injection with estriol, which had been incubated with the other tissues, are comparable with the control standard. Yet the assays following estriol incubation with liver performed under the same conditions at the same time are lower.

The author acknowledges with pleasure the participation and kind advice of Dr. E. L. Sevringhaus, Dr. F. J. Stare and Irene Stark, and gladly records his gratitude for the cooperation of Emily J. Heller.

REFERENCES

1. Lauson, H. D., C. G. Heller, J. B. Golden and E. L. Sevringhaus: *Endocrinology* 24: 35. 1939.
2. Cohen, P. P.: Inhibition of Dehydrogenases and Related Substances, p. 137, in Respiratory Enzymes by University of Wisconsin Biochemists. Burgess Publ. Co., Minneapolis, 1939.
3. Wettstein: *Helv. Chim. Acta.* 22: 250. 1939.
4. Smith, O. W., G. V. S. Smith and S. Schiller: *Endocrinology* 25: 509. 1939.
5. Golden, J. G., and E. L. Sevringhaus: *Proc. Soc. Exper. Biol. & Med.* 39: 361. 1938.
6. Pincus, G., and P. A. Zahl: *J. Gen. Physiol.* 20: 879. 1937.
7. Smith, G. V., and O. W. Smith: *Am. J. Obst. & Gynec.* 36: 769. 1938.
8. Zondek, B.: *Skand. Arch. Physiol.* 70: 133. 1934.
9. Israel, S. L., D. R. Meranze and C. G. Johnston: *Am. J. Med. Sc.* 194: 835. 1937.
10. Talbot, N. B.: *Endocrinology* 25: 601. 1939.
11. Biskind, G. R., and J. Mark.: *Bull. Johns Hopk. Hosp.* 65: 212. 1939.

FLUID AND ELECTROLYTE SHIFTS IN THE NORMAL AND ADRENALECTOMIZED RAT AFTER THE INTRAPERITONEAL INJECTION OF ISOTONIC SUGAR SOLUTIONS[1]

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THE INTRAPERITONEAL GLUCOSE injection technic of Schecter and coworkers (1) and Darrow and Yannet (2) induces, by virtue of the difference between the absorption rate of glucose from the peritoneal cavity and the diffusion of electrolyte into it, a temporary dehydration of the extracellular fluid through an increase in ascitic and intracellular volume. As pointed out by Gilman (3), the responses to such treatment resemble, in many respects, features of adrenal insufficiency. In both cases there is a hemoconcentration of the blood (3–7), a decrease in total extracellular electrolyte (3–6) and volume (8), a fall in blood pressure (3, 5), and a sensitivity to hemorrhage and like conditions of circulatory stress (3, 5). The response to glucose injections is unlike adrenal insufficiency in that there is no rise in blood potassium or non-protein nitrogen levels (4).

The adrenalectomized animal is extremely sensitive to the glucose treatment, with death usually following within a few hours after injection (5, 7, 9). The hemoconcentration induced is more rapid in onset and more severe than in the glucose injected normal animal (5, 7). Fluid absorbed from the peritoneal cavity cannot be used to redilute the concentrated blood (7). The mortality and severity of symptoms can be prevented only by large amounts of cortical hormone or salt (5, 7).

This paper, supplementary to an earlier one (7), is an attempt to describe and analyze further the abnormalities of the response of the adrenalectomized rat to intraperitoneal glucose (and sucrose) injections.

METHODS

A total of 106 normal and 95 adrenalectomized male rats of approximately 200 gm. in body weight was used. Of these, 145 were injected with 5.5% glucose and 56 with 10.0% sucrose. They were deprived of food but not water for 18 hours prior to injection. Adrenalectomy was performed about 18 hours before injection, in which interval no apparent adrenal deficiency symptoms, as indicated by hematocrit and serum electrolyte levels, had set in.

A dose of 10 cc. per 100 gm. of body weight, of the isotonic sugar solution was injected intraperitoneally, using the precautions described previously (7). When

[1] This thesis has been accepted by the Graduate School of New York University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] The author wishes to express his grateful appreciation to Dr. Robert Gaunt, under whose advisement this work was done.

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thiocyanate was used, a dose of 0.1 cc. per 100 gm. of body weight, of a 1% sodium thiocyanate solution was injected into the ventricle of the heart at the time of the sugar injections. Animals were kept in metabolism cages for urine collection. At the end of the selected time interval they were placed under light ether anesthesia and blood withdrawn from the heart. The abdominal wall was then slit and the ascitic fluid removed by free draining.

Hematocrit readings were made at selected time intervals on all rats, using quadruplicate samples of tail blood in capillary tubes. Chemical analyses were made by the following technics: chloride, Saifer and Kornblum (10); sodium, Weinbach (11); potassium, Kramer and Tisdall (12); glucose, Benedict (13); sucrose, Shaffer and Somogyi (14) after acid hydrolysis; thiocyanate, Crandall and Anderson (15). Serum electrolyte concentrations were corrected for extracellular distribution by the factor 0.95 (16).

RESULTS

On theoretical grounds, we should expect several differences in the response of the animal to intraperitoneal glucose and sucrose injections. With both solutions, diffusion of electrolyte from the extracellular fluid into the peritoneal cavity should take place rapidly, with the rate diminishing as the ascitic concentrations rise and the serum concentrations fall. At the same time, the injected sugar will diffuse into the blood and interstitial fluid. Glucose being metabolizable, its absorption will continue to completion. Sucrose, diffusing into sucrose-free blood, should show a somewhat faster absorption rate than glucose, and absorption should continue only until all parts of the extracellular fluid reach the ascitic fluid concentration, which condition will remain static until urine production begins. As the extracellular electrolyte concentrations fall, water is shifted into the intracellular compartment to restore osmotic equilibrium. Sucrose, unlike glucose, should replace to a large extent the electrolyte lost to the peritoneal cavity so that the intracellular shift should be appreciably less. The larger extracellular volume in sucrose injected rats would be reflected in less severe hemoconcentration and lower serum electrolyte concentrations. In the glucose and sucrose injected rats, the following responses were observed.

General Body State

Normal rats, glucose injected. Within 1 hour after injection, a general sluggishness and lethargy developed. The rats were irritable towards handling, the coat was roughened with hair erected. Muscular twitches were frequent by the 2nd or 3rd hour. The crisis came between the 3rd and 6th hours, followed by a gradual restoration of normal activity and alertness, complete by about the 9th hour. The recovery was always complete, and survival 100%.

Normal rats, sucrose injected. The crisis was reached about an hour earlier and recovery was more rapid.

Adrenalectomized rat, glucose injected. The onset of symptoms was more rapid than with the normal rat and the response more severe. The animals were usually prostrate from the 3rd hour. They showed no definite crisis, the symptoms became gradually more severe until death ensued. Terminal convulsions were present in about a third of the cases. Over 80% died within a 12-hour period, with the heaviest mortality between the 3rd and 7th hours after injection. The few that did survive for 12 hours usually died within the next 24, although food and water were given ad libitum.

Adrenalectomized rats, sucrose injected. The mortality was not quite so high as with glucose, and the highest frequency fell a few hours later.

Urine Production

Normal rats, glucose injected. The rats were generally anuric for the first 9 hours after

injection. The urinary output then increased rapidly and continued until all injected fluid had been excreted, usually by about the 15th hour. In 13 of 16 rats excretion had begun by the 12th hour.

Normal rats, sucrose injected. The urine flow began earlier (about the 6th hour), but continued at a slightly slower rate, so that the 12-hour total urine volumes were not appreciably different (table 1).

Adrenalectomized rats. Both glucose and sucrose injected rats remained anuric until death.

Changes in Ascitic Volume

Normal rats, glucose injected. The ascitic volume increased rapidly for the first 3 hours, when the maximum was reached. Shortly after the 6th hour the volume had receded to the point where it was once more equal to that injected. The recovery period for extracellular dehydration therefore came between the 3rd and 7th hours, which agreed well with the gross symptoms of the animal. Absorption was continued from the 6th hour at almost a linear rate. Five of 16 rats showed complete absorption by the 12th hour (table 1).

Normal rats, sucrose injected. The ascitic volumes tended to be slightly larger than with

TABLE 1. ASCITIC FLUID AND BODY WEIGHT CHANGES IN NORMAL AND ADRENALECTOMIZED RATS AFTER THE INTRAPERITONEAL INJECTIONS OF ISOTONIC GLUCOSE AND SUCROSE SOLUTIONS

	Glucose injected					Sucrose injected			
	Normal, drained at 3 hr.	Normal, drained at 6 hr.	Normal, drained at 12 hr.	Adren., drained at 3 hr.	Adren., drained at 6 hr.	Normal, drained at 6 hr.	Normal, drained at 12 hr.	Adren., drained at 6 hr.	Adren., drained at 12 hr.
Number of rats	26	29	16	29	29	16	9	19	9
Initial body weight, gm.	186	201	222	202	192	235	200	225	183
Injected sugar solution, cc./100 gm. body wt.	10	10	10	10	10	10	10	10	10
Ascitic fluid, cc./100 gm. body wt.	12.4±0.2	11.0±0.3	3.2±0.3	8.7±0.2	6.7±0.2	11.3±0.2	3.5±0.4	7.6±0.2	5.6±0.5
Final body wt. after draining, gm.	176	193	225	202	194	224	195	224	181
% change in body wt.	−5.4	−4.0	1.3	0	1.0	−5.5	−2.5	0	−1.1
Urine volume, cc./100 gm. body wt.	0	0	3.1±0.1	0	0	0.6±0.0	4.1±0.3	0	0
Injected fluid recovered as urine and ascitic fluid, %	124	110	63	87	67	119	76	76	56

glucose. Once the original volume was restored, there was no further reduction until urinary excretion began, when it decreased at a rate proportional to the amount of fluid excreted (table 1).

Adrenalectomized rats, glucose injected. There was but a short interval, if any at all, in which the ascitic volume was greater than that injected. By the 3rd hour the original volume was not only restored but reduced. The volume then regressed steadily until death.

Adrenalectomized rats, sucrose injected. The ascitic volumes tended to be larger than with glucose injections. The ascitic volume was held constant from the 6th hour, not at the original level as with the normal rats, but one approximately 60% of the original.

Sugar Absorption from the Peritoneal Cavity

Normal rats, glucose injected. Absorption was rapid for the first 3 hours, so that by the end of this time 70% of injected sugar had left the peritoneal cavity. It was then continued at a steadily decreasing rate. It was never complete by the 6th hour, but was complete in 12 of 16 rats, by the end of the 12th hour (table 2).

Normal rats, sucrose injected. Sucrose showed a steadier and somewhat faster absorption rate throughout than did glucose in the earlier hours. From the 6th hour the ascitic concentration was stabilized, and fell only as sucrose was excreted in the urine (table 2).

Adrenalectomized rats. Both glucose and sucrose absorption rates were somewhat faster than in the normal rat. Two of 21 glucose injected rats showed complete absorption by the 6th hour. Since there was no urine flow, once the minimum was reached, the ascitic sucrose concentration was held almost constant until death (table 2).

TABLE 2. SUGAR ABSORPTION IN NORMAL AND ADRENALECTOMIZED RATS AFTER INTRAPERITONEAL GLUCOSE AND SUCROSE INJECTIONS

	No. of rats	Sugar recovered %	Final sugar concentration m.-eq./L.	Sugar absorption gm./kg. body wt./hr.	Total ascitic fluid cc./100 gm. body wt.	Ascitic fluid osmotically held by sugar cc./100 gm. body wt.
Glucose Injected						
Normal, drained at 3 hr.	21	29.2±0.9	71±3.1	1.20±0.03	12.4	2.9
Normal, drained at 6 hr.	22	17.4±1.0	44±1.7	0.75±0.03	11.0	1.7
Normal, drained at 12 hr.	16	0.7±0.3	3±0.5	0.47±0.00	3.2	0.1
Adren., drained at 3 hr.	20	21.0±1.3	66±6.4	1.45±0.06	8.7	2.1
Adren., drained at 6 hr.	21	9.1±0.9	25±3.0	0.82±0.02	6.7	0.9
Sucrose Injected						
Normal, drained at 6 hr.	16	30.3±0.9	82±2.9	1.22±0.03	11.3	3.0
Normal, drained at 12 hr.	9	10.7±0.6	94±1.8	0.78±0.04	3.5	1.1
Adren., drained at 6 hr.	19	26.4±1.1	107±2.6	1.29±0.05	7.6	2.6
Adren., drained at 12 hr.	9	17.9±0.8	99±3.0	0.72±0.05	5.6	1.8

Hemoconcentration

Normal rats, glucose injected. The hematocrit level rose rapidly for the first 1½ hours and then more slowly to the 3rd hour, when the maximum was reached. From the 3rd to 12th hours the blood was slowly rediluted. When the hematocrit was of the order of 45 to 47%, seemingly a critical level, urine production began. When ascitic absorption was nearly complete the hematocrit fell to sub-initial levels until all injected fluid had been excreted (table 3).

Normal rats, sucrose injected. The hemoconcentration was less severe and recovery more rapid. This explains the earlier urine production in these animals (table 3).

Adrenalectomized rats. The hematocrit rise was more rapid and the hemoconcentration more severe than in the normal rats. Once the maximum was reached, there was no redilution until death ensued. The maximum was reached more slowly and was not so high in sucrose injected rats as in glucose injected rats (table 3).

Ascitic Fluid Electrolyte Concentrations

Normal rats, glucose injected. The electrolyte concentrations rose rapidly for the first 3 hours, and then more slowly throughout the 12-hour period. The potassium concentration rise was slower than those of sodium and chloride, which were proportionate (table 4).

Normal rats, sucrose injected. The final concentrations were always lower, a reflection of the larger extracellular volume (table 4).

TABLE 3. HEMATOCRIT READINGS ON TAIL BLOOD IN NORMAL AND ADRENALECTOMIZED RATS AFTER INTRAPERITONEAL GLUCOSE AND SUCROSE INJECTIONS

Time after injection, hr.	Glucose injected				Sucrose injected			
	Normal		Adrenalectomized		Normal		Adrenalectomized	
	No. of rats	Hematocrit %	No. of rats	Hematocrit %	No. of rats	Hematocrit %	No. of rats	Hematocrit %
0	50	42.5±0.3	55	42.4±0.3	50	42.5±0.3	55	42.4±0.3
1.5	26	50.1±0.9	27	54.5±0.8	24	49.6±0.5	21	50.4±0.4
3	48	52.8±0.4	48	57.2±0.4	26	52.2±0.4	19	53.6±0.4
6	45	49.4±0.5	44	60.5±0.7	23	45.3±0.4	14	51.9±0.3
12	20	42.7±0.9	—	—	10	40.6±0.2	11	52.0±0.4

Adrenalectomized rats, glucose injected. During the period of rapid rise, the electrolyte concentrations tended to be significantly higher than in the normal rats. The rise was considerably slowed after the 3rd hour, so that the concentrations were no longer significantly above the normal by the 6th hour. Since the ascitic volume was smaller, the total electrolyte shifted into the peritoneal cavity was always less than in the normal rat. The peak for maximum electrolyte was reached about 3 hours earlier (table 4).

Adrenalectomized rats, sucrose injected. The total ascitic electrolyte remained constant after the 6th hour, a reflection of the constant ascitic volume (table 4).

Serum Electrolyte Concentrations

Normal rats, glucose injected. Sodium and chloride concentrations fell rapidly for the first 3 hours, and then more slowly for the next three. Shortly after the 6th hour, the serum concentrations were lower than the ascitic fluid concentrations and electrolyte could be shifted back into the blood. From this time, therefore, serum concentrations showed a gradual rise. Potassium showed a complex behavior. Since it entered the ascitic fluid more slowly than did the other electrolytes, for the first 3 hours, when extracellular concentration was severe, serum potassium was actually concentrated above the initial level. Later the concentration showed a sharp drop. Since the serum concentrations were always higher than the ascitic fluid concentrations, the data seemingly indicates that all serum potassium was not freely diffusible (cf. Peters, 16). Both a serum potassium rise (6) and fall (4) have been reported in the normal animal injected with glucose. On the basis of the above findings, these results are merely reflections of different time intervals after injection (table 4).

Normal rats, sucrose injected. Serum electrolyte concentrations fell to a lower level than in the glucose injected rats. Equilibrium between serum and ascitic fluid was reached in less time (table 4).

Adrenalectomized rats. Sodium and chloride concentrations fell at a much more rapid rate than in the normal rat for the first 3 hours. By the 3rd hour, or about 4 hours earlier than in the normal rat, the ascitic fluid concentrations reached serum levels and electrolyte was shifted back into the blood. The serum concentrations, therefore, rose from the 3rd hour, so that by the 6th hour they were no longer significantly different from the still falling concentrations in the normal rat. The tendency was always toward slightly lower levels in the adrenalectomized rat. The sodium fall was proportionately greater than the chloride. The serum potassium response was similar to that of the normal rat. Although there was wide variation, the concentration was usually below that of the normal rat at all time intervals (table 4).

Extracellular Fluid Dehydration

The final extracellular fluid volumes were estimated by 4 different methods: (*a*), determination of the amount of fluid available for the dilution of injected thiocyanate, with the volume of red blood cell water deducted; (*b*) by determination of the fraction of body fluid through which injected sucrose had diffused; (*c*) assuming parallel changes in blood and extracellular fluid, by calculation from the initial and final hematocrit levels (no corrections were made for the swelling of the red blood cell, since the erythrocyte does not swell as much in hypotonic solutions as might be expected (6, 17, 18) and because the error involved would not be serious in the ranges used); (*d*), by dividing the difference between the initial total extracellular electrolyte and the total ascitic electrolyte by the final extracellular electrolyte concentrations.

Normal rats. There was no appreciable nor consistent difference between the volumes obtained from the thiocyanate technic and those from hematocrit readings, so that the extracellular fluid was dehydrated to an extent almost parallel to that of the blood. In the earlier hours, the volumes derived from electrolyte measurements tended to be slightly lower than those from thiocyanate measurement but the difference was later lost. The volumes from sucrose measurement tended to be lower than those from thiocyanate. Perhaps this is evidence that sucrose does not penetrate all body membranes permeable to thiocyanate (table 5).

TABLE 4. ASCITIC FLUID AND SERUM ELECTROLYTE CONCENTRATIONS IN NORMAL AND ADRENALECTOMIZED RATS AFTER INTRAPERITONEAL GLUCOSE AND SUCROSE INJECTIONS

	Controls	Glucose injected					Sucrose injected			
		Normal, drained at 3 hr.	Normal, drained at 6 hr.	Normal, drained at 12 hr.	Adren., drained at 3 hr.	Adren., drained at 6 hr.	Normal, drained at 6 hr.	Normal, drained at 12 hr.	Adren., drained at 6 hr.	Adren., drained at 12 hr.
No. of animals	18	29	28	16	28	29	16	29	18	9
Ascitic Cl/m.—eq./L.		77.6±0.8	92.9±0.8	97.8±1.0	86.5±1.6	93.8±1.5	83.9±0.9	92.7±0.8	81.9±0.2	79.2±0.9
Ascitic Cl, total m.—eq.		0.9622	1.0219	0.3150	0.7602	0.6419	0.9481	0.3244	0.5824	0.4435
Serum Cl, m.—eq./L.	105.6±0.5	96.0±0.8	91.7±1.1	93.2±0.8	85.9±1.4	91.4±1.0	80.4±1.2	89.2±1.1	78.1±1.0	75.1±0.9
Ascitic Cl/Serum Cl		0.71	1.01	1.05	1.01	1.03	1.04	1.04	1.05	1.04
Ascitic Na, m.—eq./L.		104.8±1.3	121.9±1.8	127.2±1.2	112.3±1.9	120.4±1.7	107.4±1.7	125.3±1.8	103.5±1.4	99.2±2.4
Ascitic Na, total m.—eq.		1.2471	1.3409	0.4070	0.9935	0.8067	1.0659	0.4035	0.7866	0.5655
Serum Na, m.—eq./L.	142.1±1.0	127.7±1.4	120.5±1.9	125.6±1.2	112.2±1.8	118.6±1.3	107.2±1.3	123.3±1.5	101.6±1.8	97.5±2.0
Ascitic Na/serum Na		0.82	1.01	1.01	1.00	1.01	1.00	1.02	1.01	1.02
Ascitic K, m.—eq./L.		2.4±0.2	3.0±0.1	4.3±0.3	2.6±0.4	3.5±0.1	3.8±0.2	4.5±0.2	4.3±0.3	4.4±0.3
Ascitic K, total m.—eq.		0.0298	0.0330	0.0133	0.0229	0.0234	0.0429	0.0158	0.0327	0.0246
Serum K, m.—eq./L.	5.9±0.3	6.3±0.2	5.8±0.2	5.3±0.3	5.6±0.3	5.0±0.2	4.7±0.2	5.5±1.3	4.6±0.2	5.4±0.4
Ascitic K/serum K		0.38	0.52	0.81	0.46	0.70	0.81	0.82	0.93	0.82

Normal rats, glucose injected. The extracellular fluid was dehydrated in the first 3 hours by a shift of 2.4 cc. per 100 gm. of body weight into the peritoneal cavity, and 3.9 cc. into the intracellular compartment. For the next 3 hours, fluid absorbed from the peritoneal cavity was held in the extracellular compartment, the intracellular volume remaining unchanged. Not until electrolyte was shifted out of the ascitic fluid did the intracellular volume decrease. The whole of the absorbed fluid was therefore retained in the extracellular compartment, and was reflected by hematocrit levels.

Normal rats, sucrose injected. Only 1 cc. per 100 gm. of body weight was shifted from extracellular to intracellular compartments. The extracellular fluid was therefore more dilute than in the glucose-injected rats by some 3 cc., or 10% of the whole extracellular volume. This difference explains the lower hematocrit levels and earlier urine flow.

Adrenalectomized rats. The values for extracellular volumes tended to fall into two groups. Those derived from thiocyanate and sucrose measurements, and that calculated from

TABLE 5. FINAL EXTRACELLULAR (PLUS ASCITIC FLUID) VOLUMES, IN CC. PER 100 GM. BODY WEIGHT, IN NORMAL AND ADRENALECTOMIZED RATS AFTER INTRAPERITONEAL GLUCOSE AND SUCROSE INJECTION

	Hematocrit	Thiocyanate	Cl	Na	K	Sucrose	Average	Ascitic fluid	True extracellular fluid	Intracellular fluid
Glucose Injected										
Normal, drained at 3 hr.	35.8	36.3	34.8	34.6	34.7		35.2	12.4	22.8	42.5
Normal, drained at 6 hr.	36.0	35.0	34.8	33.7	35.6		35.0	11.0	24.0	42.7
Normal, drained at 12 hr.	35.2	34.3	34.3	34.4	34.6		34.6	6.3	31.5	39.9
Adren., drained at 3 hr.	30.5	31.3	37.1	35.8	36.3		34.2	8.8	25.4	43.5
Adren., drained at 6 hr.	27.1	26.2	33.7	34.6	35.2		31.4	6.7	24.7	46.3
Sucrose Injected										
Normal, drained at 6 hr.	38.6	40.1	38.6	38.6	38.8	37.3	38.7	11.3	27.4	39.0
Normal, drained at 12 hr.	33.4	32.7	36.0	33.0	32.0	32.6	33.3	7.6	29.8	40.3
Adren., drained at 6 hr.	29.7	34.1	39.5	41.2	38.6	32.9	36.0	7.6	28.4	41.7
Adren., drained at 12 hr.	29.4	32.5	40.8	42.4	32.5	31.2	34.8	5.6	29.2	42.9

hematocrit levels indicated a greater extracellular dehydration than in the normal rat. Since the hematocrit value was always the lowest, vascular dehydration tended to be more severe than the extracellular. Volumes derived from electrolyte measurements indicated an extracellular volume greater than in the normal rat.

DISCUSSION

The two most outstanding differences in the response of the adrenalectomized rat to intraperitoneal sugar solution injections from that of the normal were the invariably smaller ascitic volumes and the high mortality. The smaller volume might in part be due to the faster sugar absorption rate. Yet, as shown in table 2, the residual sugar accounts for but a small part of the tonicity of the ascitic fluid. Since the difference was not influenced by the substitution of sucrose for glucose, a possible derangement of carbohydrate metabolism in the adrenalectomized animal played no part. The fact that the ascitic volume in the sucrose injected adrenalectomized rats was held at only 60% of the expected value seems evidence that in electrolyte behavior and not sugar absorption rate lies the key to the ascitic volume differences.

The adrenalectomized rat showed higher ascitic fluid and lower serum electrolyte concentrations than did the normal. The ascitic concentration difference was a reflection of the volume difference. The lower serum values are difficult to explain, for they were present in the face of a much smaller shift of total electrolyte into the peri-

toneal cavity. Accompanying this smaller electrolyte shift was a smaller water shift, so that the ascitic volumes were less, and equilibrium between ascitic fluid and serum was reached in less time in the adrenalectomized rat.

Both the high mortality and the lower serum electrolyte concentrations in the adrenalectomized rat seem correlated in some way with vascular dehydration. The hemoconcentration was more rapid in onset and more severe than in the normal rat, and was not relieved by the absorption of fluid from the peritoneal cavity. This intense dehydration explains the anuria in the adrenalectomized rats. The time of death seems to bear no relation to the amount of fluid absorbed from or the electrolyte lost to the peritoneal cavity. It is not clearly related to the extracellular volume as measured by thiocyanate. It was never seen unless the hematocrit level was 50% or greater, and usually, but not always, the hematocrit level was an accurate index of the rapidity of the approach of death. Yet the mere degree of hemoconcentrations is not the essential factor, for normal rats readily recovered from a concentration in the range of those found in dying adrenalectomized rats.

With a smaller amount of total electrolyte shifted to the peritoneal cavity and lower serum concentrations in the adrenalectomized rat, it seems clear that only a fraction of the total extracellular electrolyte and fluid was being diluted by the injected fluid in these animals. Some 55% of the initial extracellular fluid, in showing the fall from initial to final concentrations, would contribute the measured amount of electrolyte to the peritoneal cavity. After the third hour, the equilibrium time in the adrenalectomized rat, electrolyte was shifted from the ascitic fluid into 73% of the original extracellular fluid. This volume, attained but gradually, was held fairly constant until death. The size of the 'active' fraction of the extracellular fluid was somewhat larger in the sucrose injected rats. With both solutions it showed quite reasonable accord with the total extracellular volume measured by thiocyanate and by sucrose. It was considerably smaller than that derived from total electrolyte measurements. In other words, it would seem that about 25% of the total initial extracellular electrolyte was not involved in the shifts induced in this treatment, and is therefore 'unaccounted for.'

This 'active' fraction of the extracellular fluid must be enclosed by a barrier more or less impervious to electrolyte transfer. It will allow the passage of water, at least away from the active portion, for the blood and related fluid become progressively more concentrated despite absorption of fluid from the peritoneal cavity. The barrier is impassable to injected thiocyanate and sucrose.

Two interpretations of adrenal insufficiency have been advanced which lend some insight into the nature of these reported responses in the adrenalectomized rat. Swingle and coworkers (19) could not account for all fluid and electrolyte originally present in the extracellular fluid of the adrenalectomized animal, and postulated a transfer of electrolyte into the intracellular compartment. The 'active' fraction described above would then be the true final extracellular fluid, and the non-active, the fraction which had entered the intracellular compartment. In view of the reported electrolyte response, this idea is an attractive one. There are, however, several defects which cannot be satisfactorily explained. If chloride entered the cell, it is not clear why the thiocyanate ion should be barred. Also, Harrison and Darrow (9) found no evidence from tissue analyses of such a shift. Data from tissues taken from the rats used here have not proved conclusive. There was an indubitable total water gain in all tissues analyzed in the adrenalectomized rats, but it was not clear that this gain was a reflection of an electrolyte shift and not merely a reflection of a larger extracellular volume.

Swingle and coworkers (20) and Harrison and Darrow (9) have drawn attention

to a possible enhancement of the interstitial fluid volume at the expense of the blood in the adrenalectomized animal. If we assume a 'shock-like' capillary dilatation, another interpretation presents itself. If stasis were present in a sufficient number of capillaries so that some 25% of the total extracellular fluid was blocked from active participation in the fluid and electrolyte circulation required in such a crucial experiment, then most of the features reported here would be explicable. Thiocyanate and sucrose would not enter the blocked areas and would indicate a too small extracellular volume. Electrolyte transfer from one region to another would be impeded, so that serum concentrations would indicate a large fraction of the total initial extracellular electrolyte 'unaccounted for.' The blocked capillaries would present little impedance towards the transfer of water, so that fluid absorbed from the peritoneal cavity would leave the blood and fluid in equilibrium, for the electrolyte rich 'blocked areas.' The slow and progressive increase in the size of the 'active' fraction would then be understandable, as would also the fact that much more fluid is in equilibrium with the blood in the sucrose injected rats, where the hemoconcentration is slower in onset and less severe.

SUMMARY

The response of 106 normal and 95 adrenalectomized rats to intraperitoneal injections of isotonic solutions of glucose and sucrose was studied.

The substitution of sucrose for glucose did not alter the basic nature of the response, but it did afford a less drastic treatment, with less severe concentration of the blood and extracellular fluid, earlier recovery, earlier initiation of urine flow, and more rapid attainment of equilibrium between ascitic fluid and blood.

Survival in the normal rat was 100% and recovery always complete. Adrenalectomized rats were soon prostrate, and mortality was of the order of 80%.

The ascitic fluid volume in the adrenalectomized rat was always much less than in the normal. This difference contributed toward a more rapid absorption of the injected sugar, higher ascitic electrolyte concentrations, and more rapid attainment of equilibrium between ascitic fluid and blood. The difference is believed to be due to a much smaller total shift of electrolyte into the peritoneal cavity in the adrenalectomized rat.

The hemoconcentration of the blood showed a direct relationship to changes in ascitic volume in the normal rat. It showed no such relationship in the adrenalectomized rat, but was more rapid in onset, more severe, and showed no relief despite the absorption of fluid from the peritoneal cavity.

The extracellular fluid of the normal rat dehydrated to an extent almost parallel to that of the blood. External symptoms and time of urine flow can be interpreted on the basis of extracellular fluid volume changes.

Estimates from thiocyanate and sucrose determinations indicated a greater dehydration of extracellular fluid in the adrenalectomized rat than in the normal. Volumes derived from electrolyte measurements indicated a more dilute extracellular fluid. On this basis, some 25% of the initial total extracellular electrolyte in the adrenalectomized rat is "unaccounted for."

Possible interpretations of the data, in light of analogous changes in adrenal insufficiency, are discussed.

REFERENCES

1. Schecter, A. J., M. K. Cary, A. L. Carpentieri and D. C. Darrow: *Am. J. Dis. Child.* 46: 1015. 1933.
2. Darrow, D. C., and H. Yannet: *J. Clin. Investigation* 14: 266. 1935; 15: 419. 1936.
3. Gilman, A.: *Am. J. Physiol.* 108: 662. 1934.

4. Harrop, G. A.: *Bull. Johns Hopkins Hosp.* 59: 11. 1936.
5. Swingle, W. W., W. M. Parkins and A. R. Taylor: *Am. J. Physiol.* 116: 430. 1936.
6. Robinson, E. J., and A. H. Hegnauer: *J. Biol. Chem.* 116: 779. 1936.
7. Gaunt, R., J. W. Remington and M. Schweizer: *Am. J. Physiol.* 120: 532. 1937.
8. Gregersen, M. I., and G. W. Thorn: *Am. J. Physiol.* 119: 320. 1937.
9. Harrison, H. E., and D. C. Darrow: *J. Clin. Investigation* 17: 77. 1938.
10. Saifer, A., and M. Kornblum: *J. Biol. Chem.* 112: 117. 1935.
11. Weinbach, A. P.: *J. Biol. Chem.* 110: 95. 1935.
12. Kramer, B., and F. F. Tisdall: *J. Biol. Chem.* 46: 339. 1921.
13. Benedict, S. R.: *J. A. M. A.* 51: 1193. 1911.
14. Shaffer, R. A., and M. Somogyi: *J. Biol. Chem.* 100: 695. 1933.
15. Crandall, L. A. Jr., and M. X. Anderson: *Am. J. Digest. Dis. & Nutrition* 1: 419. 1934.
16. Peters, J. P.: Body Water. Charles C Thomas, Springfield, 1935.
17. Yannet, H., D. C. Darrow and M. K. Cary: *J. Biol. Chem.* 112: 477. 1935.
18. Macleod, J., and E. Ponder: *J. Physiol.* 77: 181. 1933.
19. Swingle, W. W., W. M. Parkins, A. R. Taylor and H. W. Hays: *Am. J. Physiol.* 116: 438. 1936.
20. Swingle, W. W., W. M. Parkins, A. R. Taylor and H. W. Hays: *Am. J. Physiol.* 124: 22. 1938; 123: 659. 1938.

BLOOD ADRENALIN LEVELS DURING INSULIN SHOCK TREATMENTS FOR SCHIZOPHRENIA[1]

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PROMINENT among the changing symptoms observed during insulin shock are those arising from the autonomic nervous system. The fluctuating pupil, dilating and constricting alternately, may become small and fixed at the end of deep coma. The high blood pressure, with increasing pulse pressure, extreme sweating, salivation, gooseflesh and flushing of the skin have been often described. Gellhorn (1) said "It may be concluded that the various treatments used in schizophrenia are effective because they produce, by a profound alteration in the metabolism of the central nervous system, a sufficiently strong and lasting excitation of the sympathetic nervous system and thereby bring about a far-reaching reorganization of all mental processes." Sakel (2) spoke of the "sudden transitory compensatory adrenaline mobilization (?) during early insulin shock." Cameron (3) wrote "we are making a study of the effects of adrenalin injections upon the mentation of normal subjects. This is a further offshoot of our preliminary findings to the effect that one of the end results of insulin treatment is to intensify the adrenalin reactions of the individual." Others have suggested that there is a battle between the orthosympathetic and parasympathetic systems with a predominance of the parasympathetics after prolonged coma. It therefore appeared that the actual estimation of the amount of adrenalin in the blood during insulin shock therapy might throw some interesting light on the state of the autonomic nervous system during this treatment.

METHOD

After surveying the available technics used for the determination of the adrenalin in the blood (4–9), it was decided that the method of Shaw (8) was most practicable and useful for our purposes. This is a colorimetric test, a sensitive variation of the Whitehorn (9) technic.

A protein-free filtrate of blood is fractionally treated with a specially prepared $Al(OH)_3$ which adsorbs glutathiones and probably other substances at pH 2 and adrenalin at pH 8 to 8.5. Arsenomolybdic acid is reduced by the adrenalin thus separated out to form a blue color, whose intensity we determine on the photelometer. This is compared with the color given by standard adrenalin solutions.

All patients tested for this series were schizophrenics undergoing regular hypo-

[1] This study was financed through the National Committee for Mental Hygiene from funds granted by the Committee on Research in Dementia Praecox founded by the Supreme Council, 33rd Degree Scottish Rite, Northern Masonic Jurisdiction, U.S.A.

We also gratefully acknowledge the unfailing encouragement and support of Dr. E. A. Baber, Superintendent of Longview State Hospital, and the helpful advice of Professor Shiro Tashiro of the Department of Biochemistry of the University of Cincinnati, College of Medicine, in carrying out this work.

glycemic treatment. Five cc. of blood was obtained by a 20-gage needle inserted into the arm veins. This blood was immediately shaken with 5 cc. 20% trichloroacetic acid. The first specimen was obtained at 6 A.M., before the injection of insulin. Hourly samples were then taken quickly and with care to disturb the patient as little as possible. Some of the tests were accompanied by blood pressure readings and blood sugar tests for comparison, but so much handling seemed to prevent deep shock from developing.

The fifth test was taken just before termination of the shock and the sixth about an hour later, after the patient was quite alert and up, walking about.

RESULTS

In all cases studied so far the adrenalin values ranged from 0 to 200 γ per 100 cc. of blood. We were unable to test for less than 1/10 γ per cc. (1 part in 10 million) and

TABLE 1. DUPLICATE BLOOD ADRENALIN DETERMINATIONS

Blood adrenalin	Blood adrenalin	Blood adrenalin	Blood adrenalin
(1) γ/100 cc.	(2) γ/100 cc.	(1) γ/100 cc.	(2) γ/100 cc.
56	60	0	0
54	46	0	0
120	116	0	0
56	56	0	0
32	32	20	20
28	26	20	10
44	36	15	8
56	56	0	0
84	95	0	0
40	38	38	44
12	14	0	0
0	0	22	26
26	24	72	62
20	18	88	88
30	28	34	30
40	36	26	24
54	54	52	52
22	32	30	32
44	48	10	10
58	68	2	0
2	7	72	72
24	25	20	20
27	27		
$\frac{\text{Difference}}{\text{Sigma}} = .21$ Duplicates correlate $+.977$		$\pm .005$	

our variation in individual cases on whom duplicates were run was rarely more than ½ γ (table 1).

Table 2 gives the results in 10 shock periods, figures expressed in plus and negative values, taking the pre-injection determination as zero. This table enables us to make several deductions of interest. It is seen that within the first 2 hours after injection the values for blood adrenalin either remained stationary or showed a very slight rise or fall. After 3 hours, however, there was a definite tendency toward an increase, although a few exceptions appeared. In the 4th hour the tendency to rise was much greater and more consistent. There were no cases in this group which did not show this rise. After termination all of the values were lower, although the amounts noted were not as significant as were the pre-termination values.

Table 3 (showing the progress of a patient during insulin shock, together with the blood sugar and blood adrenalin values) gives a very clear idea of this relationship.

Not all cases studied ran the same course as shown by the cases in table 2. One patient who failed to arouse half an hour after gavage, still showed a high adrenalin level at this time. Immediately after an intravenous injection of 100 cc. of 50% glucose, however, his blood adrenalin value had dropped. We have found in similar

TABLE 2. SHIFT IN ADRENALIN VALUES DURING INSULIN SHOCK

(Expressed as plus or negative values, γ/100 cc. blood, pre-injection value is taken as zero.)

Patient	6 A.M.		7 A.M.	8 A.M.	9 A.M.	10 A.M.	11:30 A.M. (1 hr. after termination)
	Actual value	Before injection					
E. M.	(105)	0	−35	−20	− 3	38	32
C. F.	(117)	0	10	−27	3	51	−31
B. K	(87)	0	0	5	43	88	45
T. J.	(69)	0	− 4	21	41	51	−22
J. Ž.	(76)	0	4	− 3	24	52	−29
W. K.	(80)	0	−11	5	17	25	−45
B. R.	(80)	0	− 4	15	25	50	−25
B. R.	(102)	0	− 4	0	−22	43	−22
A. K.	(114)	0	14	15	−22	18	
L. B.	(130)	0	30	15	0	30	−40
Total			0	26	106	446	137
Average			0	2.6	10.6	44.6	15.2

TABLE 3. BLOOD SUGAR AND ADRENALIN DURING INSULIN SHOCK

Patient: C. F. *Treatment No.* 37 *Insulin:* 30 u at 6:20 A.M. *Date* 2/25/39

	6 A.M. (Before injection.)	7 A.M.	8 A.M.	9 A.M.	10 A.M.	11:30 A.M. (1 hr. after termination)
Temperature:	98°			92°	90°	
Sleeping →						
Sweating →						
Twitching →						
Exophthalmos						
Stupor →						
Coma →						
Pos. Babinski →						
Salivation →						
Mg. sugar per 100 cc. blood:	90	76	26	28	30	194
γ adrenalin per 100 cc. blood:	117	127	90	120	160	76

cases that the failure to awaken after gavage may be associated with the delayed absorption of sugar. Unfortunately, in this case no blood sugar determinations were obtained, but the immediate awakening of the patient after intravenous injection suggests that failure of absorption was present.

Another young man was quiet and responded at all times during the hypoglycemic treatment, never becoming comatose. He did not display the typical adrenalin rise, although his blood sugar curve dropped to about the same point as in our coma-

tose cases. It is interesting to note, moreover, that the blood pressure in this case increased during the post-absorptive hours in spite of absence of signs of excitement or shock.

DISCUSSION

We have reason to believe that the error in this method, as carried out by us, is great, although carefully applied by a single investigator. Studies are being made to detect the reasons for the inconsistencies encountered and thus enhance the quantitative value of the test. Despite this crudity, however, it seems that the level of the blood adrenalin, as determined by the method of Shaw, shows a definite rise during therapeutic hypoglycemia. The presence of this increase after the period of excitement seems to rule out the possibility that it is due to motor activity. This is consistent with the findings reported by Cannon et al. (10). The maintenance of hypoglycemia without increased adrenalin and absence of coma was noted in one case. We were interested to observe that the blood adrenalin values have a definite relation to the stages of insulin shock as described by one of us (11). Whether this adrenalin increase is merely an accompanying symptom of hypoglycemia due to a suppression of cortical inhibition of the sympathetic mechanism (12) or is an essential feature of therapeutic insulin shock is uncertain. Studies of adrenalin values during other forms of shock treatments, which might throw some light on this matter, will be reported at a later date.

SUMMARY

Blood adrenalin values, as estimated by the Shaw modification of the Whitehorn test for adrenalin, show a consistent rise during the deep stages of insulin coma, with a drop after termination.

Failure to arouse from sugar administered by gavage was accompanied in one patient by a continued high blood adrenalin which was diminished following the intravenous glucose which immediately roused the patient.

The adrenalin in one case was not seen to rise with the drop in blood sugar and there was an absence of symptoms of coma.

Further studies of blood adrenalin during shock treatments for schizophrenia, with an improvement in the consistency of the test, are indicated.

REFERENCES

1. Gellhorn, E.: J.A.M.A. 110: 1433. 1938.
2. Sakel, M.: *Nerv. & Ment. Dis.* 62: 136. 1938.
3. Cameron, D. E.: Annual Report of the Commissioner of Mental Diseases, Massachusetts, Gardner State Hospital Printing Plant, 1938.
4. DeSilva, J. L.: *Biochem. J.* 31: 2171. 1937.
5. Devine, J.: *Biochem. J.* 31: 545. 1937.
6. Rogoff, J. M.: *Proc. Soc. Exper. Biol. & Med.* 36: 441. 1937.
7. Rogoff, J. M., and E. Marcus: J.A.M.A. 110: 2127. 1938.
8. Shaw, F. H.: *Biochem. J.* 32: 19. 1938.
9. Whitehorn, J. C.: *J. Biol. Chem.* 108: 633. 1935.
10. Cannon, W. B., and A. Rosenblueth: Autonomic Neuro-Effector Systems, New York, Macmillan Co. 1937.
11. Goldman, D.: *Am. J. Psychiat.* 110: 317. 1938.
12. Gerard, R. W.: *Arch. Neurol. & Psychiat.* 40: 985. 1938.

FURTHER STUDIES ON THE ANDROMIMETIC FUNCTION OF THE IMMATURE MALE RAT ADRENAL[1]

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IN THE IMMATURE male rat, castration does not cause immediate atrophy of the prostate. Price (1) demonstrated that following prepuberal castration, the prostate develops normally and maintains a functional status for a definite period. The earlier the animal is castrated, the longer the period of maintenance. Howard (2) found the same phenomenon in the mouse and also confirmed Price's findings in the rat. Howard suggested that an andromimetic activity of the immature adrenal might be responsible for the maintenance of functional prostates in the absence of the testes. Davidson and Moon (3) found that adrenocorticotropic substance administered to immature castrate male rats caused hypertrophy of the adrenal cortex. This hypertrophy was apparently associated with the production of an androgenic substance since the prostates of these rats were stimulated as indicated by gross size and histological picture.

In a recent report the present authors (4) offered direct evidence of the andromimetic capacity of the immature rat adrenal. Immature male rats were castrated at 16 days; part were adrenalectomized at 21 days and all were killed at 26 days. The ventral prostates were examined microscopically for evidence of function. In each of the 18 adrenalectomized castrates the prostate showed marked atrophy consisting of decrease in acinar diameter, alteration in the general character of the epithelium, apparent increase in the amount of interacinar tissue as well as a complete absence of 'clear areas.' These clear areas in the epithelial cells of the normal gland appear distal to the basal nuclei and have been established by Moore, Price and Gallagher (5) as indicators of functional activity. The prostates of the plain castrates, while not identical in every case with normal prostates, showed evidence of androgenic stimulation in that clear areas were present in 9 out of 13 animals. In an additional group of 6 non-castrate animals adrenalectomized at 21 days the ventral prostates were functional. This latter finding was considered as proof that the regression of the prostates in the adrenalectomized castrates was not a non-specific expression of adrenal insufficiency.

Howard (6), using a similar procedure, has also demonstrated that the adrenal is the effective agent in the maintenance of the seminal vesicle in the immature castrate mouse. More recently Gersh and Grollman (7) published the results of some experiments from which they concluded that the adrenal of the immature castrate rat does not have an andromimetic function. Since this conclusion is incompatible with the results of our previous experiments, it was decided to repeat and extend our work, evaluating our results on a quantitative as well as a qualitative basis.

Price's findings indicate that the maintenance of prostatic function in the absence of the testes decreased with increasing age. Inasmuch as the first group of experiments involved only one age group (26 days), the present experiments were extended to include older groups, 31, 36, and 41 days of age.

[1] Supported in part by a grant from the Josiah Macy Jr. Foundation.

PROCEDURE

A total of 168 immature male rats was divided into 4 main groups. The procedure is detailed for group 1 only. At 16 days, approximately half the animals in this group were castrated; at 21 days half of the castrate animals and half of the intact animals were adrenalectomized. Thus 4 equivalent sub-groups were established: (*a*) adrenalectomized castrates, (*b*) castrates, (*c*) adrenalectomized animals and (*d*) normals. At 26 days, all the animals were killed. In the remaining 3 main groups the procedure was the same except that the age at which adrenalectomy was performed was advanced 5 days for each group. Thus, in group 2, the animals were castrated at 16 days, but adrenalectomized at 26 days and killed at 36 days; in group 4 adrenalectomy was performed at 36 days and the animals were killed at 41 days.

At autopsy the ventral prostates were removed. These glands in the rat, particularly in the immature animal, are discrete, readily isolated structures. Partial fixation in situ with Bouin's fluid differentiates the gland from surrounding fatty tissue and facilitates clean dissection and removal. The prostates were placed in Bouin's fluid for 24 hours, after which they were rapidly weighed on a microbalance sensitive to 0.02 mg. and then were prepared for microscopic examination.

Adrenalectomy, in all cases, was carried out under light ether anesthesia. Subsequent to this operation all the animals were given 2% NaCl solution in place of water and the regular diet of Purina fox chow was supplemented with bread. In the younger age groups the litters were allowed to remain with the mothers beyond the usual weaning age (21 days) since, in the earlier experiment, this procedure was found to reduce the mortality rate of the young animals considerably without, in any respect, altering, the results. Daily weighings indicated that the supplemented diet was adequate for maintenance inasmuch as most of the adrenalectomized animals gained some weight during the 5-day post-operative period. The average gains in the various groups, however, were somewhat less than for the normal and castrate animals.

At autopsy careful search was made for hypertrophied adrenal remnants or rests. Suspicious tissue was sectioned and examined microscopically. Cases of confirmed slips were not included in the compiled data.

QUANTITATIVE FINDINGS

The quantitative data are presented in table 1. From these data it is clear that, even in the immature rat, castration does cause some atrophy of the prostate. This atrophy is manifested by a diminution in the gross weight of the gland. In the 26-day group, the mean prostatic weight of the castrate animals is less than one-half the mean prostatic weight of the normal controls. However, removal of the adrenals as well as the testes causes a much more pronounced atrophy as indicated by the mean prostatic weight of the adrenalectomized castrates which is less than one-quarter that of the normal. The combined removal of the adrenals and testes, therefore, produces a marked degree of atrophy, whereas the removal of the testes alone produces a lesser effect. If only the adrenals are removed and the testes left intact, there is no appreciable difference in prostatic weight as shown by comparison of the mean prostatic weights for adrenalectomized and normal animals.

On the basis of mean prostatic weights alone, it might be argued that the extra diminution of the prostate subsequent to adrenalectomy may be a reflection of the general, weakened condition of the adrenalectomized animal. It is conceivable that the prostate is involved with the rest of the body in the nutritional, metabolic and hydration changes which occur after adrenalectomy. For this reason, the findings have also been presented in the form of prost. wt./body wt. ratios. In the normal animals

of this weight and age range there was a definite correlation between the prostatic weight and the body weight (determination of r for 39 animals, 0.794). The mean values obtained for the prost. wt./body wt. ratios were subjected to statistical analysis.

At 26 days of age the ratio for the adrenalectomized castrates was 54% less than the ratio for the plain castrates. This difference is statistically significant. The ratios for the adrenalectomized animals and the normal controls showed a difference of only 2% which is not significant.

At 31 days the removal of the adrenal in addition to the testes produced a difference of only 34% between the ratios for castrates and adrenalectomized castrates. Although this difference is significant, its lesser magnitude indicates that the removal of the adrenal at this age level caused some additional atrophy of the prostate, but its presence was not as effective in preventing atrophy as at the younger age level. In

TABLE I

Age at death of animal	26 days				31 days				36 days				41 days			
	Ad-Cast.	Cast.	Ad.	Norm.	Ad-Cast.	Cast.	Ad.	Norm.	Ad-Cast.	Cast.	Ad.	Norm.	Ad-Cast.	Cast.	Ad.	Norm.
No. of animals	10	12	11	12	13	12	10	9	11	10	9	9	11	9	11	9
m. body wt. gm.	40.4	42	37.2	42.7	51.4	56.8	53.1	56.3	62	66.4	61.8	65.5	65.1	74.7	60.7	72.1
m. prost. wt. mg.	6.11	13.75	25.97	29.23	5.89	9.84	42.18	43.9	5.78	7.10	41.41	49.75	5.86	5.97	44.36	51.75
m. prost. wt. / m. body wt.	157 ± 10 P.E.[1]	334 ± 38	692 ± 30	698 ± 32	114 ± 4	174 ± 7	784 ± 47	773 ± 11	92 ± 4	109 ± 8	670 ± 27	749 ± 44	91 ± 3	84 ± 5	740 ± 24	726 ± 27
% differences between mean ratios	54%[2]		2%		34%[2]		2%		12%		12%		11%		1%	

[1] Decimals eliminated.

P.E. derived by the formula $0.6745\sqrt{\frac{\Sigma \delta^2}{n(n-2)}}$

[2] Significant difference in $\frac{\text{prost wt.}}{\text{body wt.}}$ ratios between the two groups compared.

$$\text{Difference} > 4\sqrt{(\text{P.E}_{m1})^2+(\text{P.E}_{m_2})^2}$$

this group again there was no significant difference (2%) between the adrenalectomized and normal animals.

In the 36-day group, the difference between the ratios for adrenalectomized castrates and plain castrates was 12% which is the same as the difference between the adrenalectomized and normal animals of this group. Neither value is significant.

In the 41-day group also no significant difference was found between the ratios for the adrenalectomized castrates and the castrates (11%) and between the adrenalectomized and normal animals (1%). Therefore, in the 36 and 41-day groups, the adrenal has lost its andromimetic capacity as judged by the weight of the ventral prostate.

This decline in the ability of the adrenal to sustain the prostate of the castrate rat is in accord with Price's findings on the functional state of the gland after early castration. She found that the prostate retains its functional state (presence of clear areas) for a period of 30 days following castration during the first week of life, or approximately up to an age of 35 days. With later castration (16 to 21 days) the duration of the maintenance period is 10 to 20 days, which again indicates that the functional state is not found after 41 days.

QUALITATIVE FINDINGS

Since photomicrographs of the different conditions in the prostate were included in the first publication, they are not reproduced here. Table 2 represents an evaluation of the microscopic findings on a quantitative basis. Although fully aware of criticisms which, justifiably, might be directed against such a procedure, we feel that the presentation of our observations in such a form is preferable to an all-embracing but, perhaps to the reader, a meaningless statement of the histological conditions found in the prostates of the various groups. The method, furthermore, has honorable precedents in the McPhail test for progestational activity and in the Allen-Doisy vaginal smear test, or the Fluhman histological test for estrogenic activity. The system devised for the derivation of a functional rating is as follows. In the normal functioning prostate the majority of acini possess high columnar epithelium with clear areas just distal to the basal nuclei. There is a minimal amount of stroma in these functional prostates. This condition has been designated +3; the presence of clear areas in some acini with good epithelium, but absence of clear areas in the majority of acini, +2; a generally normal appearance of acini and epithelium but with no distinct clear areas +1; and a general picture of atrophy as indicated by small acini with disorganized epithelium, no clear areas and much interacinar stroma has been designated 0.

TABLE 2

	Ad-Cast.		Cast.		Ad.		Norm.	
	No. animals	Average func. rating	No. animals	Average func. rating	No. animals	Average func. rating	No. animals	Average func. rating
26 days	27[1]	0.11	22[1]	1.91	12[1]	2.95	9	3.00
31 days	9	0.00	6	0.66	10	2.95	8	2.81
36 days	9	0.00	9	0.11	7	3.00	5	2.90
41 days	5	0.00	5	0.00	3	3.00	3	3.00

[1] These groups include 18 adrenalectomized castrates, 13 castrates, and 6 adrenalectomized animals from an earlier series.

In the adrenalectomized and normal animals the mean rating for the functional status of the prostates at the different age levels varied from 2.81 to 3.00. In the castrate animals at 26 days of age the mean rating was 1.91. The range in this group was from 0 to +3; 3 of the 22 prostates were completely atrophic (0), while 4 in this group were completely functional (+3). In the older groups (31, 36 and 41 days) the functional rating for the castrates was progressively decreased until, in the 41-day group, the rating reached 0.

In the adrenalectomized castrate group at 26 days the mean rating was 0.11. Three of the 27 prostates examined were not completely atrophied in that the epithelium was still fairly high and the acini were of good size. However, no clear areas were found in any of these 3 prostates so that they may be regarded as being non-functional. The remaining 24 prostates in this group were all completely atrophic. In the remaining age groups the mean functional ratings for the adrenalectomized castrate animals were all 0.

At 26 days the prostates of the castrate animals exhibited different degrees of androgenic stimulation. The prostates of the adrenalectomized castrates at 26 days showed no indication of androgenic stimulation. At 31 days the difference between the castrates and adrenalectomized castrates was less marked than at 26 days, at 36 days the difference was still less, and at 41 days both groups exhibited complete lack

of androgenic stimulation. From these findings it is apparent that the ability of the adrenal to maintain the castrate prostate diminished with increasing age. The capacity was practically lost by 36 days and was completely lost by 41 days of age. The qualitative findings, therefore, corroborate the quantitative findings.

The agreement between the qualitative and quantitative findings is most strikingly shown in one case which was not included in the data presented. An adrenalectomized castrate animal showed considerable weight gain so that it was suspected of being incompletely adrenalectomized. At autopsy this suspicion was confirmed by the recovery of adrenal tissue. The prostate of this animal weighed 11.24 mg., which is a greater weight than was obtained for any of the other adrenalectomized castrate prostates of the same age. Microscopic examination of the prostate of this animal showed a +2 condition, i.e., some clear areas were present. The presence of a small amount of adrenal cortical tissue in this animal was sufficient to maintain a low degree of function in the prostate.

DISCUSSION

From the results of this experiment we are again forced to conclude that at a certain age in the immature male rat the adrenal, in the absence of the testes, assumes an andromimetic function. Furthermore, the waning of this capacity with increasing age has been clearly demonstrated by the experimental results. It is therefore somewhat disconcerting that the experimental results published by Gersh and Grollman (7) should lead these authors to the opposite conclusion, i.e., that the adrenal of the immature castrate male does not exhibit an andromimetic function.

Among other experiments, these workers subjected 14-day-old rats to castration and combined castration and adrenalectomy. Part of the animals were killed at 21 days and part at 28 days of age. The prostates of these animals, as well as prostates of normal controls (3 animals in each group) were compared as to average diameter of tubules, average cell height, and relative volume of the gland. No statement is made concerning the number of determinations made to provide the average values for tubular diameter and cell height. The volume determinations were derived by the "paper weight" method for one animal in each group. Utilizing these criteria, they found no difference between the adrenalectomized castrates and the castrates. However, they claim to have confirmed Price and Howard on the matter of delayed atrophy of the prostate in young rats and mice following castration. They conclude, therefore, that the absence of the adrenal gland is without effect on the development of the accessory glands "at least as far as cell height and tubular diameter are concerned" and that the "Price effect" is due to "an 'innate' potentiality for growth."

From the volume determinations the same conclusion was reached, although the values given in the table show that the prostate of the adrenalectomized castrate at 21 days was only 68% of the normal volume, while the prostate of the castrate was 86% of the normal volume. The authors apparently regarded this difference as being well within the limits of the experimental error involved, inasmuch as these values were obtained for only one animal of each type.

In addition these authors tested extracts of adrenal tissue for androgenic activity. Ten to twenty gm. of human fetal adrenals, pig fetal adrenals and x-zone bearing adrenals of mice were extracted. These extracts were tested for androgenic activity on unspecified numbers of castrated rats and capons. The results of these tests were negative. This procedure was considered to "demonstrate the absence of any *considerable* [their italics] amount of androgen in the adrenal." It is probably true that there is no considerable amount of androgen in the adrenal, but we do not believe that these authors were justified in basing such a conclusion on their experimental

results. They failed to consider the fact that the concentration of androgen is low in all tissues including the testes. According to Womack and Koch (8), extracts equivalent to 25 to 50 gm. of bull's testes must be given per day to capons to produce comb growth. The extracts used by Womack and Koch were prepared in a manner essentially similar to those of Gersh and Grollman, i.e., without "violent chemical procedure."

Gersh and Grollman also disagree with the experimental conclusions reached by Davidson and Moon (3) that the adrenals in the castrate rat may be stimulated by high doses of adrenocorticotropic substance to produce sufficient androgen to maintain the prostate in a functional state. Gersh and Grollman prefer to believe that the stimulation of the prostate by adrenocorticotropic hormone "may be explained as a direct effect of the extract on the prostate rather than as an indirect effect elicited through the adrenals." This position is untenable in that they have failed to take into consideration that Davidson (9) did not obtain an androgenic effect when adrenocorticotropic hormone was given to adrenalectomized castrate animals.

We have no evidence to submit regarding whether specialized areas of the adrenal (x-zone, andromimetic zone) produce androgenic substances or whether androgenic substances (adrenosterone and progesterone) are found in adrenal extracts only because of vigorous chemical treatment, as intimated by Gersh and Grollman. However, we feel justified in concluding that the adrenal glands in the male rats of our colony, during a limited period of their existence, are capable of producing physiologically detectable amounts of androgenic substance.

SUMMARY

Complete castration atrophy was found in the ventral prostates of 26, 31, 36 and 41-day-old adrenalectomized castrated rats. The ventral prostates were partially maintained in castrated, non-adrenalectomized animals at 26 and 31 days, but not at 36 and 41 days. In the presence of the testes adrenalectomy alone did not modify the ventral prostates at any of these ages. From these data it was concluded that, in the absense of the testes, the adrenals had an andromimetic capacity in the younger immature rat (26 and 31 days old). This capacity was lost in the older immature animals (36 and 41 days old).

REFERENCES

1. Price, D.: *Am. J. Anat.* 60: 79. 1936.
2. Howard, E.: *Am. J. Physiol.* 119: 339. 1937.
3. Davidson, C. S., and H. D. Moon: *Proc. Soc. Exper. Biol. & Med.* 35: 281. 1936.
4. Burrill, M. W., and R. R. Greene: *Proc. Soc. Exper. Biol. & Med.* 40: 327. 1939.
5. Moore, C. R., D. Price and T. F. Gallagher: *Am. J. Anat.* 45: 71. 1930.
6. Howard, E.: *Proc. Am. J. Physiol.* p. 123. 1939.
7. Gersh, I., and A. Grollman: *Am. J. Physiol.* 126: 368. 1939.
8. Womack, E. B., and F. C. Koch: *Endocrinology* 16: 267. 1932.
9. Davidson C. S.: *Proc. Soc. Exper. Biol. & Med.* 36: 703. 1937.

VARIATIONS IN THE EFFECTIVENESS OF PERCUTANEOUSLY APPLIED ANDROGENS IN THE RAT[1]

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IT IS KNOWN that estrogens are absorbed through the skin and produce systemic effects (1–5). It is fairly well established that estrogens are more effectively absorbed in the rat when applied in lipoid solvents such as benzene or alcohol than when dissolved in an oil or ointment (2–4). It has been demonstrated that in the rat or mouse non-esterified estrogens given percutaneously in alcohol are more effective than when given subcutaneously in oil (4).

Contradictory conclusions have been reached concerning the percutaneous effectiveness of the androgens. Moore, Lamar and Beck (6) state that in the rat testosterone is more effective percutaneously in ointment than subcutaneously in oil. Zondek and Sulman (7) state that testosterone is more effective in the rat subcutaneously in oil than percutaneously in alcohol. They obtained no effect with testosterone percutaneously in oil. Moore, Lamar and Beck found that testosterone in ointment is more effective percutaneously than testosterone propionate in ointment by the same route. The number of animals in the experimental groups used by these workers was very small, and their data was not statistically analyzed. In view of the discrepancies in the reported percutaneous effectiveness of androgens compared to that of estrogens, it seemed of value to test the effect of esterification and of the solvent on the percutaneous effectiveness of testosterone, using large groups of animals and subjecting the findings to statistical analysis.

PROCEDURE

Male rats weighing 150 ± 25 gm. were castrated. Treatment was started on the following day and continued daily for 21 days. One day after the last treatment the animals were killed. Using a dissecting microscope the ventral prostates and seminal vesicles were removed, freed of all extraneous tissue and weighed.

A total of 219 animals was used in these experiments. The number of animals, androgen, solvent and route of administration for each group are shown in table 1. The daily dose of androgen was 0.2 mg. and its concentration was kept uniform; 0.2 mg. was present in 0.1 cc. of the oil, lanolin and alcohol and in 0.11 cc. of the commercial ointment.

Percutaneous applications were measured from a tuberculin syringe and spread on the back of the test animal with the barrel of the syringe, the mode of application being kept as constant as possible from day to day in each animal. The hair on the back of the animals was clipped with scissors and maintained in the clipped condition.

This study included control groups of 20 untreated normals, 20 untreated castrates, and 20 castrates given daily subcutaneous injections of 0.2 mg. androgen in oil.

[1] Supported in part by a grant from the Josiah Macy, Jr. Foundation. The crystalline testosterone propionate (perandren), and the crystalline testosterone and the androgen ointments were generously furnished by Ciba Pharmaceutical Products, Inc.

The weight range of the normals was approximately the same as that of the treated animals when killed. The untreated castrates and subcutaneous controls weighed 150 ± 25 gm. at castration and were killed 22 days later.

RESULTS

The means, ranges and standard errors of the means of the body weights and ventral prostate and seminal vesicle weights are shown in table 1. Table 2 shows the differences between the mean weights of the prostates and seminal vesicles of the various groups together with the critical ratios between these obtained differences and the standard deviations of the differences.[2] Inasmuch as the treatment given the various groups differed both as to the solvents and as to the androgens used, the results will be considered as separately influenced by these factors.

TABLE 1. THE EFFECT OF 0.2 MG. TESTOSTERONE OR TESTOSTERONE PROPIONATE DAILY FOR 21 DAYS IN THE CASTRATE RAT

Solvent	Route	Animals	Ventral prostates, mg.		Seminal vesicles, mg.		Body weights, gm.	
			Means	Ranges	Means	Ranges	Means	Ranges
Testosterone Groups								
Alcohol	Percutaneous	20	259.0 ± 13.7[1]	185–395	530.8 ± 36.2	325–975	197.5 ± 4.9	155–235
Peanut oil	Percutaneous	18	145.0 ± 10.8	85–235	232.2 ± 27.6	70–470	169.4 ± 6.0	145–235
Lanolin	Percutaneous	20	139.5 ± 12.1	75–245	253.2 ± 29.7	65–515	180.0 ± 4.4	135–215
Ointment	Percutaneous	20	149.5 ± 12.8	55–245	230.0 ± 34.7	75–525	171.0 ± 3.0	145–205
Testosterone Propionate Groups								
Alcohol	Percutaneous	20	193.2 ± 10.1	110–310	307.9 ± 21.1	190–510	194.5 ± 4.0	165–235
Ointment	Percutaneous	20	86.0 ± 6.8	45–165	91.5 ± 12.0	35–185	182.5 ± 4.5	145–215
Control Groups								
Normals		20	176.0 ± 9.0	105–245	397.0 ± 30.9	105–535	181.0 ± 3.4	165–235
Untreated castrates		20	26.0 ± 1.8	15–45	61.5 ± 4.7	35–95	176.0 ± 4.3	145–205
Testosterone, peanut oil	Subcutaneous	20	189.5 ± 18.6	65–385	383.5 ± 36.4	125–765	178.0 ± 3.8	145–225
Test. prop., peanut oil	Subcutaneous	19	326.5 ± 9.8	225–420	1015.0 ± 50.6	675–1475	182.5 ± 3.9	165–225

[1] ± here signifies the standard error of the mean, indicating that the chances are 2 in 3 that the true mean lies within this range instead of the 1 in 2 chance indicated by probable error.

The Influence of the Solvent on the Effectiveness of Daily Percutaneous Administration of 0.2 mg. Testosterone

Since a critical ratio of 3 or more is indicative of a statistically significant difference between the two means being compared, it can be seen by inspection of table 2 that the percutaneous absorption of testosterone can be significantly modified by the use of different solvents. The prostates and seminal vesicles of the group receiving percutaneous testosterone in alcohol were significantly larger than those of the percutaneous oil, lanolin and ointment groups. The prostates of the alcohol group were even significantly larger than those of the control group given an equal amount of testosterone subcutaneously in oil. The seminal vesicles, while much larger than those of the subcutaneously treated animals, did not show a significant variation. The

[2] $\text{C.R.} = \frac{\text{obtained difference}}{\text{sigma of difference}}$ Obtained difference = mean 1 − mean 2. Sigma of difference = $\sqrt{(\sigma \text{ mean } 1)^2 + (\sigma \text{ mean } 2)^2}$.

TABLE 2. THE DIFFERENCES BETWEEN THE MEAN WEIGHTS OF THE PROSTATES AND SEMINAL VESICLES OF THE VARIOUS GROUPS. THE CRITICAL RATIOS BETWEEN THE OBTAINED DIFFERENCES AND THE STANDARD DEVIATIONS OF THE DIFFERENCES APPEAR IN PARENTHESES

	Testosterone alcohol percut.	Testosterone oil percut.	Testosterone lanolin percut.	Testosterone ointment percut.	Test. prop. alcohol percut.	Test prop. ointment percut.
Testosterone alcohol Percut.	p. sv.					
Testosterone oil Percut.	p. 114.0 (6.6) sv. 298.6 (6.6)					
Testosterone lanolin Percut.	p. 119.5 (6.5) sv. 277.6 (5.9)	5.5 (0.3) 21.0 (0.5)				
Testosterone ointment Percut.	p. 109.5 (5.9) sv. 300.8 (6.0)	4.5 (0.3) 2.2 (0.0)	10.0 (0.6) 23.2 (0.5)			
Test. prop. alcohol Percut.	p. 65.8 (3.9) sv. 222.8 (5.3)					
Test. prop. ointment Percut.	p. sv.			63.5 (4.4) 138.5 (3.8)	107.2 (8.8) 216.4 (8.9)	
Testosterone oil Subcut.	p. 69.5 (3.0) sv. 147.2 (2.9)	44.5 (2.1) 151.3 (3.3)	50.0 (2.2) 130.3 (2.8)	40.0 (1.8) 153.5 (3.0)		
Test. prop. oil Subcut.	p. sv.				133.3 (9.7) 707.1 (12.9)	240.5 (20.2) 923.5 (17.8)
Normal controls	p. 83.0 (5.1) sv. 133.8 (2.8)	31.0 (2.2) 164.8 (4.0)	36.5 (2.4) 143.8 (3.4)	26.5 (1.7) 167.0 (3.6)	17.2 (1.3) 89.1 (2.4)	90.0 (8.0) 305.5 (9.2)
Castrate controls	p. 233.0 (16.9) sv. 469.2 (12.9)	119.0 (9.2) 170.7 (6.1)	113.5 (9.3) 191.7 (6.4)	123.5 (9.6) 168.5 (4.8)	167.2 (16.2) 246.4 (11.4)	60.0 (8.6) 30.0 (2.3)

p.=prostate. sv.=seminal vesicles.

prostates of the alcohol group were also significantly larger than those of the untreated intact controls, although the seminal vesicles were again not sufficiently larger to establish a significant deviation.

No significant difference was found between the percutaneous effectiveness of testosterone in oil, lanolin, or ointment. Testosterone given percutaneously in these 3 solvents was less effective than subcutaneously in oil. As judged by the mean weights of the prostates of the various groups the differences are not statistically significant, but the seminal vesicles of the percutaneous oil and ointment groups were significantly lighter than those of the subcutaneous oil group. Testosterone applied percutaneously in oil, lanolin, or ointment was absorbed sufficiently to maintain the seminal vesicles as well as the prostates over the castrate level as shown by the fact that the mean organ weights of all 3 groups were significantly larger than those of the control castrates.

The Influence of the Solvent on the Effectiveness of Percutaneously Administered Testosterone Propionate

As shown in table 2 the daily percutaneous application of 0.2 mg. of testosterone propionate, like the free androgen, is more effective when dissolved in alcohol than in ointment. Both the prostates and the seminal vesicles of the alcohol group were significantly larger than those of the ointment group. However, testosterone propionate applied percutaneously in either alcohol or ointment would not maintain the prostates and vesicles in the normal state, both groups having significantly smaller prostates and vesicles than the normal controls. While the mean weights of both prostates and vesicles of the two percutaneous groups were larger than those of the castrate controls, the increase in the size of the seminal vesicles of the ointment group over the control group was not significant.

Testoterone propionate is much less efficient percutaneously than subcutaneously, as is shown by the fact that the prostates and seminal vesicles of both percutaneous groups were a great deal smaller than those of the subcutaneous testosterone propionate control group.

The Effect of Esterification on Percutaneous Absorption of Testosterone

As shown in table 2 free testosterone is absorbed through the skin in a more efficient manner than is testosterone propionate when the dose is 0.2 mg. daily. With this dosage in both alcohol and ointment the prostates and vesicles of the testosterone groups were significantly larger than those of the testosterone propionate groups.

While these experiments were in progress we were informed that this difference between percutaneous testosterone and testosterone propionate was not perceptible at higher dosage levels. Accordingly additional groups of animals were treated with 0.6 mg. testosterone in alcohol and 0.6 mg. testosterone propionate in alcohol daily. At this dosage the means of the prostates and seminal vesicles of the two groups were fairly close and no significant difference was found (table 3).

TABLE 3. THE EFFECT OF PERCUTANEOUS TESTOSTERONE AND TESTOSTERONE PROPIONATE IN ALCOHOL AT 0.6 MG. DAILY FOR 21 DAYS

	Animals	Ventral prostates		Seminal vesicles		Body weights		Prostates		Vesicles	
		Mean (σM.)	Range	Mean (σM.)	Range	Mean (σM.)	Range	Obtained difference	C.R.	Obtained difference	C.R.
Testosterone	10	372.0 (29.9)	290–570	855.0 (58.0)	625–1175	177.0 (4.7)	155–205	50.3	1.3	3.3	0.0
Test. prop.	12	321.7 (26.6)	210–510	858.3 (70.9)	525–1225	194.2 (12.1)	125–255				

DISCUSSION

This study has demonstrated that the efficiency of percutaneously applied androgens in the rat is significantly altered by the type of solvent employed. The finding that the androgen in alcohol is percutaneously more efficient than in oil or ointment is in agreement with that reported by other workers for estrogens (2, 4).

Our finding that the free testosterone is more effective than testosterone propionate when both are given percutaneously agrees with the previously reported conclusions of Moore, Lamar and Beck. We are, however, unable to confirm their findings that free testosterone in ointment is more efficient than free testosterone given subcutaneously in oil. This disagreement can, perhaps, be explained by the fact that their determinations in this regard were based on 2 animals treated percutaneously and 2 subcutaneously.

It is also possible that the use of very small groups of animals may explain the discrepancy between our findings and the observation of Zondek and Sulman that

percutaneous testosterone in alcohol is less effective than subcutaneous testosterone in oil. To reach this conclusion they used 5 animals in each experimental group.

It is our opinion that it is necessary to use large groups of animals, and to subject the results to statistical analysis to obtain valid conclusions in experiments of this type. There is a large variation in the weights of the prostates and seminal vesicles of both normal and treated rats. In the normal untreated control group, as can be noted in table 1, the ventral prostates varied from 105 to 245 mg. and the seminal vesicles from 105 to 535 mg. Similar ranges in the weights of these organs were found in each of the treated groups. In fact some of the greatest ranges in organ weights occurred in the 2 groups in which the treatment was most precise insofar as dosage was concerned, i.e., the subcutaneous testosterone and testosterone propionate groups. The large intra-group variations in organ weights is not due to variations in the body weights of the animals concerned, inasmuch as no significant correlations were found between the organ and body weights. Due to this large variation in the weights of the prostates and seminal vesicles of both normal and treated animals, the use of small experimental groups of 2 or 5 animals then, might lead to highly erroneous conclusions.

No definite explanation can be offered for the observation that percutaneous testosterone in alcohol has been found to be more efficient than testosterone propionate in alcohol at a 0.2 mg. a day dosage level, but not at 0.6 mg. a day. A possible explanation is that the 0.6 mg. dosage of both substances is above that at which a maximal response is obtained, and therefore no quantitative difference between testosterone and testosterone propionate is elicited. In this regard it may be noted that when Moore gave testosterone and testosterone propionate percutaneously in an ointment at this dosage level, testosterone was more effective than testosterone propionate. While direct comparisons between the findings in rats of one colony with those of another are perhaps not valid, it is of interest to note that the seminal vesicles of their testosterone group (584 mg.) and testosterone propionate group (220 mg.) were about the same weight as we obtained with 0.2 mg. testosterone and testosterone propionate in alcohol.

SUMMARY

Using the weight increase in the prostate and seminal vesicles of the rat as criteria, the following observations have been made.

Testosterone is more effective percutaneously in alcohol than in oil, lanolin, or ointment. Testosterone propionate is more effective percutaneously in alcohol than in ointment.

Testosterone is more efficient percutaneously in alcohol than subcutaneously in oil. It is, however, less efficient percutaneously in oil, lanolin, or ointment than subcutaneously in oil.

Testosterone propionate is less efficient percutaneously in alcohol or ointment than subcutaneously in oil.

At 0.2 mg. a day, testosterone is more effective percutaneously in alcohol or ointment than is testosterone propionate. At 0.6 mg. daily in alcohol no difference was found in the percutaneous efficiency of these two androgens.

REFERENCES

1. Zondek, B.: *Schweiz. Med. Wchnschr.* 65: 1168. 1935.
2. Zondek, B.: *Lancet* 1: 1107. 1938.
3. Jadassohn, W., E. Uehlinger and W. Zuescher.: *Klin. Wchnschr.* 16: 313. 1937.
4. Ito, M., Hajuza and T. Kon: *Zentralbl. f. Gynäk.* 61: 1094. 1937.
5. Berger, M.: *Klin. Wchnschr.* 16: 1428. 1937.
6. Moore, C. R., J. K. Lamar and N. Beck: *J.A.M.A.* 111: 11. 1938.
7. Zondek, B., and F. Sulman: *Proc. Soc. Exper. Biol. & Med.* 40: 633. 1939.

STUDIES ON THE INHIBITORY HORMONE OF THE TESTES[1]

IV. EFFECT ON THE PITUITARY, THYROID AND ADRENAL GLANDS OF THE ADULT MALE RAT

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THE PREPARATION of an extract, of bull testes, which produces inhibitory effects on the gross and microscopic appearance of the sex organs of adult male rats has been described (1, 2). In this paper we will consider the effect of this extract on the weight and microscopic appearance of the pituitary, thyroid and adrenal glands of the adult male rat.

EXPERIMENTAL PROCEDURE

The data reported herein were derived from the pituitary, thyroid and adrenal glands of the series of rats we reported on previously (1, 2). The weights of the glands and the body-organ values were obtained in the same manner as reported in the former papers except that each body-organ value was divided by 100 in order to facilitate the reading of the tables.

The tissues were fixed in formalin Zenker solution and embedded in paraffin. The thyroid and adrenal sections were cut at 10 micra and stained with hematoxylin-eosin. The pituitary sections were cut at 6 micra and stained by a modification of Heidenhain-Azan substituting acid fuchsin for the azo-carmine.[3] With this method the eosinophils stain bright red, the basophils stain sky blue, and the chromophobes take a nondescript staining.

EXPERIMENTAL RESULTS

Pituitary. Examination of the tables showed that the average body-organ value of the whole pituitary gland of the control animals was 294, whereas, the same figure for the experimental animals was 408. Therefore, the whole pituitary gland of the rats that received the extract decreased in weight, since, by this method, the larger figure designates smaller actual weight.

On examination of the sections of the anterior pituitary of the experimental animals, no morphological changes were noted in the eosinophil cells. Actual counts of these cells were not attempted because the sections were not prepared for this

[1] Aided by grants from the Committee on Scientific Research, American Medical Association, and The Eli Lilly Company, Indianapolis, Indiana.

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[3] The method is outlined as follows:

remove paraffin in xylol	Anilin blue-orange G mixture, 1 hour
95% alcohol	Orange G..... 2 gm.
80% alcohol	Anilin blue..... 0.5 gm.
70% alcohol plus iodine, 10 minutes	Acetic acid..... 8 cc.
5% sodium thiosulphate until iodine is removed	Water.....100 cc.
Running water, 10 minutes	Dilute with 2 times volume of water
1% aqueous solution acid fuchsin, 10 minutes	Rinse quickly in water
Running water, 10 minutes	Differentiate quickly in 95% alcohol
Phosphotungstic acid, 5%, for 1½ to 2 hours	Finish dehydration in absolute alcohol
	Clear in xylol and mount in gum-damar.

type of work. On the other hand, significant changes were noted in the basophil cells. They were larger and stained more deeply in the anterior pituitary of the experimental animals than in the control animals. Many of the basophils had clear vacuoles, others showed vacuolization with a fine, basophilic staining reticulum, and many had the signet ring appearance of the so-called castration cell (fig. 3). These vacuolated cells showed a marked variation in size. We found vacuolated basophils in the anterior lobe of the control animals, as did Ellison and Wolfe (3), but they were fewer in number, smaller, and more uniform in size as compared with those found in the experimental animals. We also noted a greater blood supply in the pituitaries of the experimental animals.

Thyroid. The body-organ values showed that there was some decrease in the weight of the thyroid of the experimental animals as contrasted with the control,

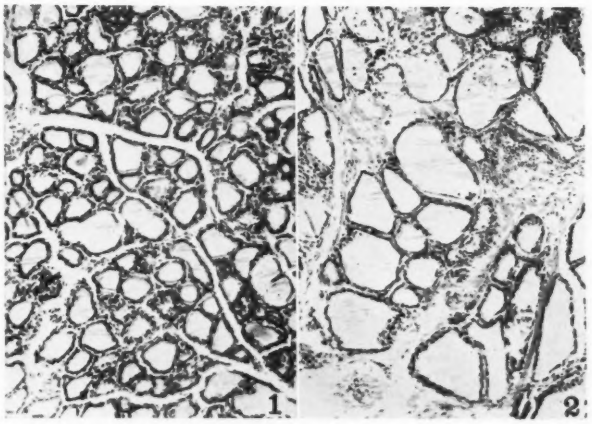

Fig. 1. Section of control thyroid, Fig. 2. Section of thyroid of rat which received 1 cc. of inhibitory hormone daily for 30 days (×67).

but it was slight. The microscopic sections, however, showed more definite differences.

The controls (fig. 1) showed the usual variation in the size of the alveoli with the largest toward the periphery of the gland. The acini were closely aligned and separated by a narrow band of connective tissue stroma. The colloid stained deeply acidophilic. Scattered outside the acini were many islands of epithelial cells without acinous formation. The epithelial cells of the acini were cuboidal in type with nuclei containing a well-defined chromatin network.

In the experimental animals the acini were more uniform in size and larger than in the control (fig. 1). There was an increase in the interacinar connective tissue and the colloid did not stain as deeply as the control. Interacinar collections of epithelial cells were present but to a lesser extent than in the controls. The acinar epithelium was flat, showed many breaks in its continuity, and the cells contained pyknotic nuclei.

Adrenal. The average body-organ value of the adrenals for the control series was 67, whereas the average for the experimental was 41. This gland, unlike the other endocrine glands in this series, showed a significant increase in weight after injection

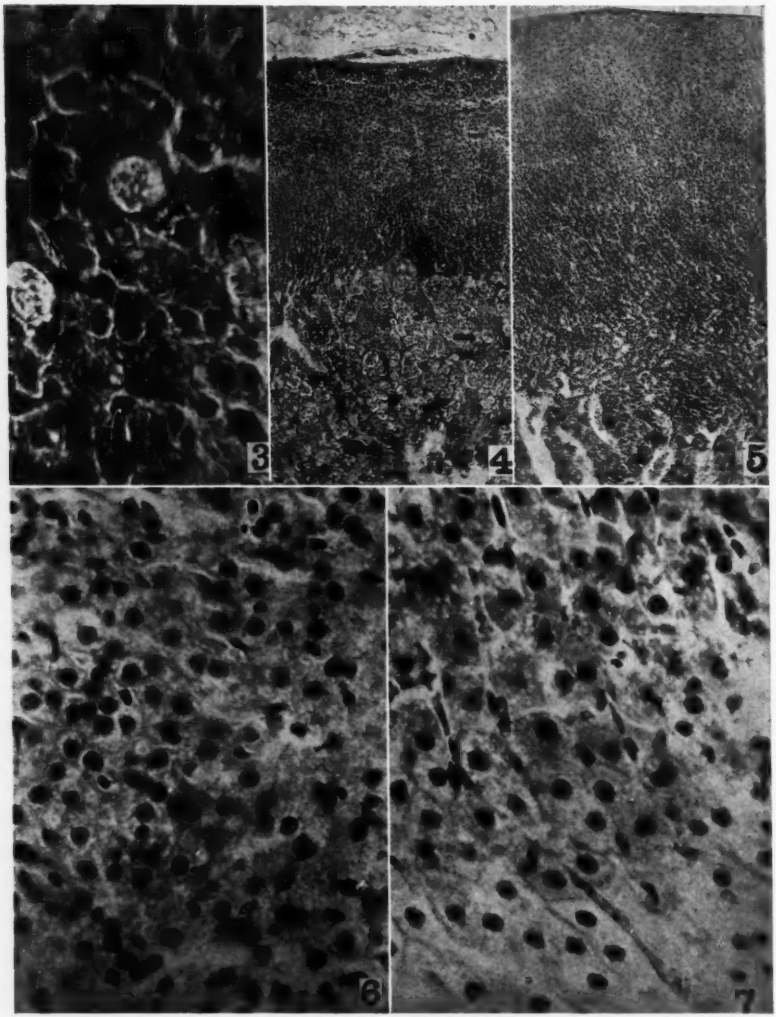

Fig. 3. SECTION OF ANTERIOR PITUITARY OF EXPERIMENTAL RAT SHOWING VACUOLATED CELLS (×285). Fig. 4. CONTROL ADRENAL (×50). Fig. 5. EXPERIMENTAL ADRENAL SHOWING HYPERTROPHY OF THE CORTEX (×50). Fig. 6. CONTROL ADRENAL SHOWING CELLS OF THE Z. FASCICULATA (×205). Fig. 7. EXPERIMENTAL ADRENAL SHOWING HYPERTROPHY OF THE CELLS OF THE Z. FASCICULATA (×205).

of the extract. A study of the microscopic sections revealed that this increase in weight was due to an increase in width of the cortex, the result of hypertrophy of the zona fasciculata and reticularis (fig. 4, 5). This was evidenced by both an increase in the size of the cells and in the number of cells. Although both processes were evident in the two zones, the increase in the size of the cell was seen mainly in the fasciculata (fig. 6, 7), whereas the increase in the number of cells was to be noted especially in the reticularis. The cells of the zona reticularis were not as uni-

TABLE 1. CONTROLS

Animal No.	Animal wt.	Thyroid wt.	Pituitary wt.	Adrenal wt.	Body organ value (100) Thyroid	Pituitary	Adrenal
	gm.	gm.	gm.	gm.			
39	325	0.017	0.010	0.054	191	325	60
57	288	0.018	0.010	0.031	160	290	92
58	290	0.025	0.009	0.058	116	322	50
73	246	0.013	0.009	0.042	188	273	58
74	196	0.014	0.006	0.028	140	326	70
75	280	0.020	0.009	0.029	140	311	96
76	220	0.018	0.008	0.039	122	275	56
77	244	0.017	0.009	0.036	143	271	67
78	244	0.035	0.009	0.041	70	271	59
79	252	0.038	0.009	0.042	66	280	60
			Average		134	294	67

formly arranged in the experimental adrenals as in the controls. In the former, bands of cells were seen crisscrossing each other, leaving islands in which no cellular structure was seen. The cytoplasm in the cortex of the experimental adrenals stained lighter than the control and showed hydropic degeneration. The lipoid content was decreased in the fasciculate zone but was not noticeably changed in the zona reticularis. The normal demarcation zone between the glomerulosa and the fasciculata, described by Hall and Korenchevsky (4), could not be demonstrated in all of the control animals but it was uniformly absent in the experimental animals.

DISCUSSION AND CONCLUSIONS

Extreme caution must be exercised in interpreting the effects of a sex hormone on the endocrine organs, an observation that can be readily understood from the many

TABLE 2. EXPERIMENTAL ANIMALS

Animal No.	Animal wt.	Thyroid wt.	Pituitary wt.	Adrenal wt.	Body organ value (100) Thyroid	Pituitary	Adrenal
	gm.	gm.	gm.	gm.			
62	296	0.021	0.009	0.091	140	308	32
63	194	0.009	0.008	0.084	215	242	23
64	212	0.012	0.007	0.041	176	302	51
65	240	0.011	0.008	0.062	218	300	38
66	200	0.017	0.009	0.062	117	222	32
67	330	0.022	0.009	0.073	150	366	45
68	280	0.014	0.009	0.084	200	311	33
69	312	0.018	0.010	0.062	173	312	50
26	180	0.020	0.001	0.039	90	1800	46
25	184	0.047	0.006	0.037	40	306	49
21	170	0.023	0.005	0.053	74	340	32
20	240	0.026	0.006	0.036	92	400	66
15	200	0.015	0.006	0.032	133	332	65
13	160	0.012		0.047	133		34
70	260	0.021	0.008	0.073	124	325	35
71	246	0.013	0.007	0.075	189	351	32
72	250	0.011	0.008	0.066	227	312	37
			Average		147	408	41

diverse reports seen in the literature. The results obtained depend upon the quantity of hormone used, the sex, species and age of the animal, and whether the animal is castrated, hypophysectomized or intact. In comparing our results with the results obtained with estrone and testosterone, we wish to emphasize that we have limited ourselves to the adult, intact, male white rat.

Estrone and testosterone are lipoid-soluble, chemically related to cholesterol, and have many effects in common. Although we know little, as yet, of the chemical nature of the extract used in this work, it is water-soluble and therefore differs from the male and female hormones mentioned above.

It is fairly well agreed upon by workers in this field that the injection of estrogenic substances into normal, adult rats causes an increase in the weight of the pituitary and adrenal glands, and a decrease in weight of the testes, seminal vesicles and prostate (5, 6, 7). Korenchevsky and Dennison (8) found, in the normal rat, that estrone in doses of 10 I.U. to 180 I.U. for 7 to 43 days did not cause any marked histological change in the testes. There was a slight decrease in the weight of the seminal vesicle due to a decrease in the amount of the secretion, with a slight increase in the fibrous tissue, but there was no constant or marked change in the epithelial cells. The weight of the prostate decreased but the histological structure did not differ from that of the normal. The adrenal was hypertrophied and there was a definite increase in the weight of the pituitary. The thyroid showed no histological change and very little variation in weight from the normal. These same authors (9) found that larger doses of estrone produced a marked depressing effect on the weight of all the male sex organs of the rat. These effects are mediated primarily through a suppression of the pituitary.

Testosterone caused a slight decrease in the weight of the testes, with a slight decrease in the size of the testicular tubules, but spermatogenesis was present (10). This action was believed to be due to a depressing effect on the anterior pituitary (11–14) since in the hypophysectomized animal, testosterone has a stimulating effect on the testes (15, 16). The suppression of the testicular hormone would lead to atrophy of the secondary sex organs but this suppression is counterbalanced by the direct stimulation of the injected male hormone. Therefore, the secondary sex organs were hypertrophied. The pituitary of all the testosterone injected rats were of normal weight with the exception of 2 senile rats which developed adenomata. The adrenals and thyroid showed no change. Moore and Price (17) found that testosterone propionate caused retardation and injury to the testes of young normal male rats, but that this effect declined with the increased age of the rat. Large doses caused less effect than small doses. This was probably due to the substitution of enough hormone to replace the damaged hormone output of the animal.

Crooke and Korenchevsky (18) reported that no histological effect was produced in the pituitary glands of normal male rats by the injection of synthetic testicular hormones. It is agreed by almost all workers that the administration of estrone to the normal male rat causes an increase in the weight of the pituitary. They found that the injection of even larger doses of estrone had no influence on the histological appearance of the pituitary in spite of the increased weight of the gland. Mazer, Israel and Alpers (19) found no histological changes in the pituitary of rats which had received 100 R.U. estrone for 100 days. Weil and Zondek (20) feel that the failure to find such changes after the injection of estrone was due to the relatively small dose used. They found their results to be qualitatively similar to those of Wolfe and his coworkers (21, 22, 23). With doses larger than 120,000 M.U. estrone injected over a period of 9 to 12 weeks, the enlargement of the pituitary gland increased by double

or more, an increase in the vascularity of the gland, and a degranulation of the chromophobe cells.

From this review it can be seen that the results obtained by the injection of this extract of bull testes approximate the effects of estrone on the endocrine organs of the rat with one important difference. In this series of animals there was a definite decrease in the weight of the pituitary gland. From the results obtained in this work, we feel that the changes were initiated by an inhibitory effect on the anterior lobe of the pituitary, which secondarily involved the testicle, or else the extract directly antagonized the lipoid-soluble male hormone with secondary castration effects on the anterior pituitary. The decrease in weight of the pituitary, however, tends to make us lean towards the former explanation. The changes in the accessory sex organs followed the changes in the testes as did also the changes in the adrenal. Since hypophysectomy causes atrophy of the adrenal, as well as the other endocrine organs, and since the changes in the pituitary included only the basophil cells of the pituitary, this work would tend to show that the adrenal is not under the control of the basophil secretion.

SUMMARY

The effect of an extract of bull testes with inhibitory effects was demonstrated on the pituitary, thyroid and adrenal glands of adult, normal, male rats.

The pituitary of the experimental animals decreased in weight, and the anterior lobe showed an increase in size of the basophil cells, many of which were vacuolated.

The thyroid was insignificantly changed in weight but showed, in the microscopic sections, a more uniform size of acini, larger acini, paler staining colloid, flat epithelium, increase in connective tissue stroma and pyknotic changes in the nuclei.

The adrenals showed an increase in weight due to hypertrophy and hyperplasia in the zonae fasciculata and reticularis.

These changes were compared with those of estrone and testosterone under similar conditions, and their significance discussed.

REFERENCES

1. Vidgoff, B., R. Hill, H. Vehrs and R. Kubin: *Endocrinology* 25: 391. 1939.
2. Vidgoff, B., and R. Hill: *Endocrinology*, to be published.
3. Ellison, E. T., and J. M. Wolfe: *Endocrinology* 19: 160. 1935.
4. Hall, K., and V. Korenchevsky: *Nature* 140: 318. 1937.
5. McEuen, C. S., H. Selye and J. B. Collip: *Lancet* 1: 775. 1936.
6. del Castillo, E. B., and R. Sammartino: *Compt. rend. Soc. de Biol.* 129: 870. 1938.
7. Noble, R. L.: *Lancet* 2: 192. 1938.
8. Korenchevsky, V., and M. Dennison: *J. Path. Bact.* 41: 323. 1935.
9. Korenchevsky, V., and M. Dennison: *Biochem. J.* 28: 1474. 1934.
10. Korenchevsky, V., and K. Hall: *Brit. M. J.* 1: 4. 1939.
11. Moore, C. R., and D. Price: *Endocrinology* 21: 313. 1937.
12. Hertz, R., and R. K. Meyer: *Endocrinology* 21: 756. 1937.
13. Hamilton, J. B., and J. M. Wolfe: *Endocrinology* 22: 360. 1938.
14. Cutuly, E., and E. C. Cutuly: *Endocrinology* 22: 264; 568. 1938.
15. Cutuly, E., D. R. McCullagh and E. Cutuly: *Am. J. Physiol.* 121: 786. 1938.
16. Hamilton, J. B., and S. L. Leonard: *Anat. Rec.* 71: 105. 1938.
17. Moore, C. R., and D. Price: *Anat. Rec.* 71: 59. 1938.
18. Crooke, A. C., and V. Korenchevsky: *Proc. Roy. Soc. Med.* 28: 72. 1935.
19. Mazer, C., S. L. Israel and B. J. Alpers: *Endocrinology* 20: 753. 1936.
20. Weil, A., and B. Zondek: *Endocrinology* 25: 114. 1939.
21. Wolfe, J. M., and D. Phelps: *Proc. Soc. Exper. Biol. & Med.* 32: 1305, 1935.
22. Wolfe, J. M., and C. S. Chadwick: *Endocrinology* 20: 503. 1936.
23. Wolfe, J. M., and A. W. Wright: *Endocrinology* 23: 210. 1938.

THE EFFECTS OF TESTOSTERONE PROPIONATE ON THE OVARIECTOMIZED MATURE RAT

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THE EFFECTS of testosterone propionate n the ovariectomized rat have been extensively investigated by Korenchevsky and his associates (1, 2). These authors determined the effects of 3 dosage levels of the substance (0.167, 0.7, 1.5 mg.) administered daily for 21 days. At all 3 dosage levels, they observed a marked increase in the weight of the uterus, vagina and preputial glands. The adrenal glands, on the other hand, became smaller and the thymus showed a greater degree of involution than is ordinarily seen in the ovariectomized rat. The hypophysis showed no significant change in weight.

Of particular interest were their observations on microscopic structure of the endometrium and vaginal mucosa. The latter showed various stages of mucification at all dosage levels. At the 2 highest dosage levels (0.7, 1.5 mg.), the uterine mucosa assumed a lace-like appearance similar to that seen in this species during pregnancy. At the lowest dosage level (0.167 mg.) progestational changes were, however, not observed. At a similar dosage level (0.2 mg.) no progestational changes were observed by McKeown and Zuckerman (3) even in the estrogen-primed ovariectomized rat, though it is known that estrogen priming enhances the effect of testosterone on the endometrium (2).

The ability of testosterone propionate to produce progestational changes at certain dosage levels was also demonstrated in the estrogen-primed ovariectomized rabbit (4, 5). However, in two estrogen-primed ovariectomized monkeys, daily 10 mg. doses of testosterone propionate given for 13 and 63 days failed to produce pregravid uterine changes (6).

The experiment herein described was undertaken to determine the effect of length of treatment with testosterone propionate at a given dosage level on the organs of the ovariectomized rat.

EXPERIMENTAL

Forty-four adult rats were ovariectomized and each immediately injected with 2 R.U. of estradiol. The same dose of estradiol was repeated in 3 days. Two days later, when all of the animals were in full vaginal estrus, testosterone propionate[1] administration was started. The preliminary estrogen treatment was given in order to facilitate the production of a progestational reaction.

Twenty-two of the animals were given daily injections of 2 mg. of testosterone propionate in 0.2 cc. of sesame oil. The remaining 22 animals, serving as controls, received daily injections of 0.2 cc. of sesame oil. Vaginal smears were studied daily. Four or five animals of each group were killed at intervals of 3, 8, 15, 23 and 29 days. The uterine horns, preputial glands, hypophysis and adrenal glands of each animal were removed, dissected free of extraneous tissue and weighed in the fresh state. Sections of the uterus and vagina were studied histologically.

[1] The authors are indebted to Doctors Gregory Stragnell and Erwin Schwenk of the Schering Corporation for the generous supply of testosterone propionate (oreton) used in these experiments.

RESULTS

The weights of the organs of the treated and control animals are given in table 1. For comparison the organ weights of a group of intact female rats from another experiment are included. The progressive changes in organ weight of the control and treated animals are shown in figures 1 to 3. The curves were fitted to the values by simple inspection.

Uterus. Since a portion of each uterine horn is removed during ovariectomy in order to insure complete castration, the weight of the uterine horns is somewhat less

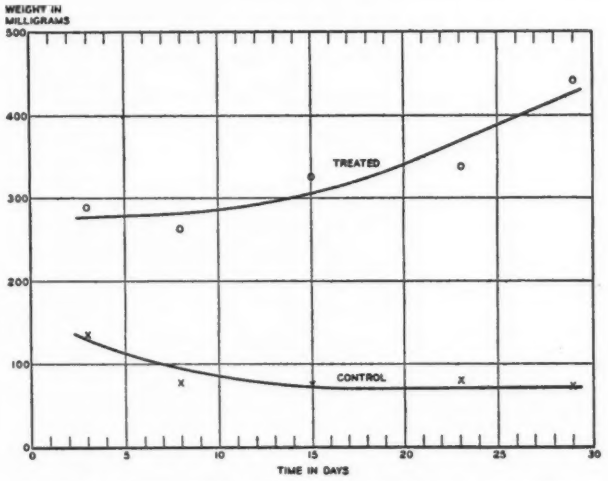

Fig. 1. Influence of testosterone propionate on the growth of the uterus of the ovariectomized mature rat. Note the early and progressive increase in the uterine weight of the treated animals, and the decrease in uterine weight incident to ovariectomy in the control animals.

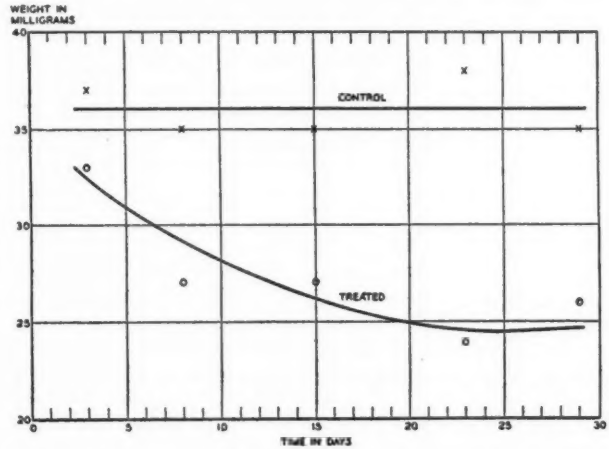

Fig. 2. Loss of weight of the adrenal glands incident to testosterone propionate administration in the ovariectomized mature rat as compared with that of controls.

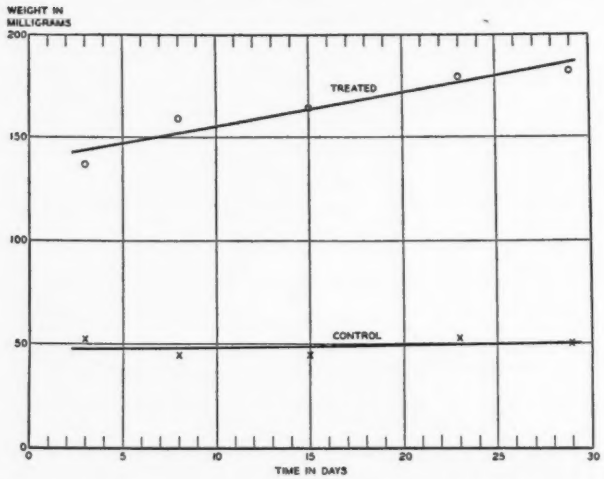

Fig. 3. INFLUENCE OF TESTOSTERONE PROPIONATE ON THE GROWTH OF THE PREPUTIAL GLANDS OF THE OVARIECTOMIZED MATURE RAT. Note the early and progressive increase in preputial gland weight in the treated animals as compared with that of controls.

than it would otherwise be. This, however, pertains alike to the treated and control animals.

As seen in table 1, the control uterine horns atrophied rapidly after castration and withdrawal of estrogen treatment, reaching the basal level of 77 mg. within 10 days after the last estrogen injection. No further atrophy was observed in the groups of animals killed at intervals during the remaining 21 days. The weight of the uterine horns of the testosterone treated animals, on the other hand, increased progressively throughout the experiment and had apparently not reached a limiting value at the end of 29 days (table 1). At that time the uterine horns had an average weight of 442 mg. which is 6 times that of the controls and nearly twice that of the intact adult rat (table 1).

TABLE 1. AVERAGE WEIGHT OF ORGANS

	Intact rats	Ovariectomized control rats					Ovariectomized treated rats				
		Days of treatment					Days of treatment				
		3	8	15	23	29	3	8	15	23	29
Number of rats	20	4	4	4	5	5	5	4	5	4	4
Body weight at autopsy (gm.)	161	154	149	152	178	172	146	147	143	149	155
Uterine horns (mg.)	272	135	77	75	79	73	288	262	325	337	442
Adrenal glands (mg.)	40	37	35	35	38	35	33	27	27	24	29
Preputial glands (mg.)	80	52	45	45	52	50	137	159	164	179	182
Hypophysis (mg.)	8.9	7.5	7.4	8.1	10.7	9.7	7.8	7.4	6.9	7.4	7.0

The histologic changes in the endometrium were of particular interest. The endometriums of animals which had received testosterone for 3 days showed little or no modification. After 8 days of treatment, progestational changes began to appear. Within 15 days after the first injection of testosterone propionate a frank progestational reaction, comparable to that current in the empty horn of the pregnant

rat uterus, was present (fig. 5). The progestational reactions in the animals treated for 23 to 29 days were no greater (fig. 6). Cystic changes were present in some. It is evident from the foregoing that testosterone propionate has the combined effects of estrogen and progestin on the uterus of the ovariectomized rat, evoking both pronounced uterine growth and a progestational reaction.

Vagina. During the entire period of testosterone treatment the vaginal smears of both control and treated animals showed a diestrous phase. The vaginas of the treated animals were grossly larger than those of the controls. The vaginal mucosa showed a high degree of mucification, with extreme vacuolization in some, a phenomenon also observed in intact testosterone treated rats (2, 7).

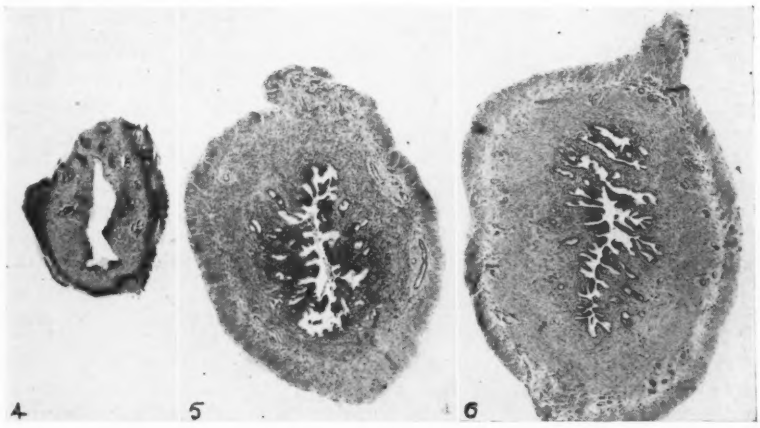

Fig. 4. UTERINE HORN OF OVARIECTOMIZED CONTROL RAT (×18). Note the atrophy which reached a maximum in 10 days after the second dose of estrogen. Fig. 5. UTERINE HORN OF 15-DAY TREATED OVARIECTOMIZED RAT (×18). Note the size of the uterus and the lace-like appearance of the endometrium which is comparable to that of the empty uterine horn of a pregnant rat. Fig. 6. UTERINE HORN OF 29-DAY TREATED OVARIECTOMIZED RAT (×18). Note further uterine growth without, however, accentuation of the progestational effect.

Adrenal glands. It has been shown that castration of female rats produces no significant change in the weight of the adrenal glands (1). This is confirmed by a study of the adrenal gland weights of the untreated ovariectomized animals in the experiment (table 1). Those of the testosterone treated ovariectomized animals, however, showed a decrease in weight (table 1) comparable to that observed in testosterone treated intact animals (7).

Hypophysis. The weight of the hypophysis appeared to increase in the control animals during the course of the experiment (table 1), while that of the treated animals remained relatively constant. However, because the differences are small, the number of observations few, and the errors involved in dissection and weighing of so small an organ great, the observation is of doubtful significance.

Preputial glands. The preputial glands of the untreated castrated animals weighed 50 mg. on the 29th day which is somewhat less than the weight of those of intact animals. The preputial glands of the testosterone treated animals, on the other hand, were greatly increased in weight. They reached a weight of 182 mg. on the 29th day. The greatest increment of growth occurred within the first 3 days (table 1).

Other tissues. Gross observation indicated that the thymus of the testosterone treated animal involuted at a more rapid rate than that of the control ovariectomized animal. The nipples of the testosterone treated animals were larger and more erythematous than those of controls.

DISCUSSION

Our observation that testosterone propionate in adequate dosage has a dual effect on the uterus of the rat, when the ovaries are eliminated as a factor, confirm those of Korenchevsky and his associates (1, 2). It produces pronounced growth of the organ as does estrogen and evokes a pregnancy-like reaction in the endometrium as does progestin.

The principal objective of the experiment herein described was to determine the length of treatment necessary to evoke these uterine effects. By killing groups of animals at various intervals it was found that uterine growth appears early and is progressive throughout the course of treatment. A full progestational effect is, however, not evident before the fifteenth day of treatment. Thereafter, the progestational development remains stationary with a tendency to cyst formation in some instances.

Although testosterone propionate produces uterine and vaginal growth, as does estrogen, it does not evoke cornification of the vaginal epithelium. On the contrary, it produces extreme degrees of mucification.

The decrease in weight of the adrenal gland noted in testosterone treated ovariectomized rats also occurs in the intact female rat (7) and is presumably due to pituitary inhibition.

SUMMARY

A survey of the literature and an analysis of the experiment herein described indicate that testosterone propionate exerts both an estrogenic and a progestin effect on the uterus of the ovariectomized rat, producing marked growth of the organ and a pregnancy-like state of the endometrium. By killing groups of animals after 3, 8, 15, 23 and 29 days of treatment, it was found that the estrogenic effect of testosterone propionate on the uterus appeared early and was progressive throughout the duration of treatment. The progestin effect, on the other hand, did not appear before the eighth day of treatment and was not complete before the fifteenth day. Longer treatment maintained the progestational appearance of the endometrium but produced cystic changes therein.

Despite vaginal growth, cornification of the epithelium was not noted. Instead, marked mucification of the vaginal epithelium occurred. The preputial glands were enormously enlarged as a result of testosterone treatment. This seems to be a distinctive effect of the substance. The adrenal glands were smaller in the treated than in the control animals, presumably through inhibition of pituitary function.

REFERENCES

1. Korenchevsky, V., M. Dennison and M. Eldridge: *Biochem. J.* 31: 475. 1937.
2. Korenchevsky, V., and K. Hall: *J. Path. & Bact.* 45: 681. 1937
3. McKeown, T., and S. Zuckerman: *Proc. Royal Soc. London. s. B.* 124: 362. 1937.
4. Klein, M., and A. S. Parkes: *Proc. Royal Soc. London s. B.* 121: 574. 1937.
5. Robson, J. M.: *Quart. J. Exper. Physiol.* 26: 355. 1937.
6. Hartman, C. G.: *Proc. Soc. Exper. Biol. & Med.* 37: 87. 1937.
7. Mazer, M., and C. Mazer: *Endocrinology* 24: 175. 1939.

ADOLESCENCE OF MACAQUES[1]

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THIS STUDY concerns the sexual maturation of primates, from the menarche, or the first menstruation, to the first ovulation and subsequent pregravid endometrial development. Data demonstrating the fact that menarche and first ovulation are not concurrent events have been accumulating steadily from observations on mice, rats, the rhesus monkey, the Cebus monkey, the chimpanzee, the gorilla and human females (reviewed by Ashley-Montagu, 1). Hartman (2, 3) was the first to apply the term "adolescence" to the period of sterility separating the two events.

Hartman (2) reported his observations on 15 immature female monkeys (*Macacus rhesus*) in which he found that the average weight for the 15 animals at first menses was 3350 gm, while the average weight at the time of the first conception was 5000 gm. with the lowest weight at 4370 gm. This difference in weight indicated that an interval of time, which he estimated at one year, had elapsed between menarche and first conception despite many menstrual cycles and frequent matings. Sterility due to anovulatory cycles Hartman (3) attributed to "endocrine instability" which is a consequence of the "stair-case phenomenon" The "staircase phenomenon" results (according to Hartman) from the necessity for the gradual physiological adjustment of the body to one phase of the reproductive cycle before another can begin. This phenomenon may be observed after lactation or any long period of sexual inactivity.

Zuckerman (4) made similar observations on the baboon and the rhesus monkey. In his discussion of fertility in monkeys and man, Zuckerman (5) defined both puberty and menopause as sterile periods, characterized by inefficient function of the endocrine system. He suggested that:

> Only the first phase of the full ovulation cycle is experienced for a long period after the process of reproductive maturation (puberty) sets in both in man and monkeys. [Zuckerman, p. 39. (5)]

Although information on the sexual development of the chimpanzee is still limited by the small number of cases under observation, the high correlation coefficient of the data indicates its reliability. Yerkes (6) reported an interval of sterility of one year and one month in the captive-born chimpanzee, *Cuba*. *Cuba* reached menarche at the age of 7 yr. 4 mo. and conceived at 8 yr. 5 mo. It is interesting that Elder (7) observed the first menstruation in the chimpanzee, *Soda*, at the age of 8 yr. 4½ mo. *Soda* was, therefore, a year and a half month older than Yerkes' *Cuba* at menarche, but she conceived at 9 yr. 2¼ mo. after just a ten months sterility interval. In both of these animals the sexual skin phenomenon preceded menarche by 2 or 3 months.

The main purpose of the present study is to describe the period between menarche and conception, the period of adolescent sterility, in terms of ovarian function. As evidences of ovarian activity we observed the sex skin, the vagina, uterus and ovaries by bimanual palpation, and studied vaginal smears. Incidentally it was possible to correlate the vaginal smear method (8) as a technic of estimating ovarian activity with the other methods. Hartman had already made such an attempt (9) with inconclusive results except for the correlation between total desquamation and ovulation. In our hands, it seemed that the vaginal smears showed a cycle more clearly, and were more

[1] This work was in part supported by a grant from the Rockefeller Foundation to the Brush Foundation.
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[3] Rockefeller Foundation Fellow.

sensitive to small changes than the daily bimanual palpations, the reliability of which is indubitable.

Six of the 7 animals observed were immature at the beginning. Their immaturity was manifested by the presence of a high percentage of cells from the basal layer of the vaginal mucosa in the smears both before and at the time of menarche as well as by our failure to find any cyclical ovarian changes by rectal palpation. The initial immaturity of the animals was indicated also by the fact that the sexual skin was observed to develop through all of the initial phases as described by Zuckerman (10). The inception of the sexual skin as an excessive edema of the central pubic folds was seen in 5 animals; preceding the first menstruation in 3 monkeys (*monkey 426, 430, 431*), but succeeding menarche in the other two (*monkey 428, 432*). The weight at the time of the first menstruation also indicated the youth of the animals. The average weight at menarche was 3400 gm.; the weights ranging from 2950 gm. to 3800 gm. At conception, however, the weights were still the same as at the time of menarche in the 3 animals which conceived during the second cycle (*monkey 430, 431, 432*) and in *monkey 426* which conceived during the fourth cycle there had been a loss in weight. The average weight at the time of conception was 3460 gm.

Because the exact age of these animals was unknown, a monthly approximation of the status of their growth and development was made by Dr. Carl Francis employing x-ray assessment of the skeleton. Due to the fact that there are as yet no standards for the age pattern of the skeletal growth of monkeys the assessments were made on the basis of the atlas of the normal development of children (11). The assessments are, therefore, comparative in terms of age of equivalent development of human females. At menarche two monkeys had reached the comparative age of 12 yr. 10 mo.; two 12 yr. 11 mo.; and two 13 yr. 1 mo. The first ovulation with the resulting pregnancy occurred in 4 of the 6 adolescents at the relative ages of 13 yr. 6 mo., 13 yr. 1 mo., 12 yr. 10 mo. and 13 yr. 2 mo.

MATERIALS AND METHODS

The prereproductive cycles and accompanying phenomena of 6 adolescent and one adult female monkeys (*Macacus rhesus*) were observed during the past 9 months, September, 1938 through May, 1939. While there are many studies describing adolescence in primates, none was directed explicitly toward the description of menarche and the early ovulations, and in none was the vaginal smear method used. With the description by Hartman (12) of the bimanual rectal palpation technic for rhesus monkeys, an objective method for determining the time of ovulation was established. Unfortunately, for obvious anatomic reasons, this technic is only exceptionally applicable to woman. In addition to palpating the developing ovarian follicles, it was desirable to correlate vaginal smear changes so that inferences could be made in those species for which palpation methods are impossible. We were unable to employ the bioelectric method (13, 14), because of interfering muscle potentials and because of the time element. It was impossible to obtain basal rectal temperatures or basal metabolic rates because of their instability in monkeys.

Smears were taken and palpations performed on all of the monkeys 6 days a week unless they were pregnant or ill. Sunday was not omitted for any animal in the preovulatory phase. The male monkey was segregated from the female monkeys. Mating was permitted only during a 24-hour period when the ovulatory stage was indicated by smears and palpations. This procedure served as a further check on the time of ovulation. In this study 1300 slides were examined and evaluated, approximately 1100 palpations performed.

DATA AND DISCUSSION

Although menarche was the first dramatic event in the sexual maturation of the rhesus monkey, many prophetic and important changes occurred during the premenarche. Activity of the sexual skin often preceded the first menstruation. Zuckerman (4, 15) found this to be inevitably true for both monkeys and baboons. Three of the macaques (*monkey 426, 430, 431*) observed in this study developed the swelling of the central pubic folds before the menarche. During the premenarche the vaginal smears of the 6 animals contained many cells from the basal layers of the mucosa. Approximately 10 to 15 days before menarche the smears began to show a progressively greater proportion of supra basal cells indicating an increased thickness of the vaginal mucosa. The degree of cornification gradually increased until a few days before the onset of the flow when there was a sudden regression. The increased cornification is not necessarily the result of the growth and maturation of a follicle (as none was palpable), but might simply have indicated general ovarian activity.

Menarche, the first menstrual flow, was observed in all 6 adolescent macaques. The table presents the weight and x-ray assessment of the monkeys at the time of the menarche. It is evident that the animals closely approximated Hartman's (2) average figure of 3350 gm. for the weight at menarche; and that the comparative age of the monkeys correlated highly with the average age of 13 years at the time of menarche for girls in this climate (16, 17).

No. of monkey	Menarche	Wt. at menarche, gm.	X-ray assessment at menarche[1]
426	October, 1938	3500	12 yr. 11 mo.
427	January, 1939	2950	12 yr. 10 mo.
428	October, 1938	3290	12 yr. 11 mo.
430	September, 1938	3490	13 yr. 1 mo.
431	September, 1938	3800	13 yr. 1 mo.
432	October, 1938	3400	12 yr. 10 mo.

[1] Giving the comparative ages of the monkeys based on the standards for the skeletal development of normal children, due to the lack of an age pattern for the skeletal growth of the macaque.

Menarche was not heralded by an excessive swelling of the central pubic folds in *monkeys 428* and *432*. Instead the sexual skin development began during the first intermenstrual period. *Monkey 427*, the most immature of the group failed to exhibit a sexual skin either before or after the isolated menstrual period which she had had in January. During the first menstrual cycle the edema of the central region spread to the peripheral areas of the buttocks and thighs and down one-third of the tail in *monkey 426, 430* and *431*, the animals which had shown sex skin development in premenarche.

The hyper-edematous condition of the sexual skin area during the first cycle did not necessarily reflect complete follicular maturation resulting in the expulsion of an ovum. On the contrary, the first menstrual cycles were unfailingly anovulatory. Small follicles were continually and persistently palpable on the ovaries of each of the monkeys during the intermenstrual period. The vaginal smears showed very high cornification. Nevertheless, ovulation failed to occur during this initial cycle, and probably for the same reason that in the smears the cornification remained constantly high. There was no aggregation and folding of the cells; there was very little desquamation; all of which indicated a lack of luteinization. These observations tend to confirm Zuckerman's generalization (5) that follicle development begins before corpus luteum development.

The length of the period of sterility following menarche differed for the individual monkeys but in only two instances did it approach a year, the sterility period for adolescent macaques as estimated by Hartman (2). In the 5 months since her menarche in January, *monkey 427* had never ovulated. The vaginal smears for the entire period exhibited few cyclic changes; epithelial cells of the basal layer of the mucosa, degenerating leucocytes and mucus were its chief elements. On palpation the ovaries were persistently small, firm and smooth. The uterus remained tiny and atonic. *Monkey 428* underwent the sexual rhythm for 3 months but the cycles were irregular and no follicles ruptured. The first cycle was 44 days long. During this time many follicles were palpated on the right ovary but none exceeded 3 mm. in diameter and none ruptured. Immediately after the second 2-day menstrual flow on November 16th and 17th a follicle started to develop on the right ovary. It had receded, however, by the 6th day of the cycle. Six days later another follicle was palpable on the same ovary but it also regressed. Mating was allowed over a 24-hour period when the follicle was at its maximum growth in each instance although the cornification was not quite complete at these times. Conception failed to occur, the follicles receded instead of rupturing. In the course of the very long cycle (52 days) edema of the pubic folds and of the peripheral areas was almost ever present. Following the third menstruation, in the first week in January, the vaginal mucosa regressed and the smears showed many basal cells. A fourth menstrual period occurred from January 20th through the 24th but no follicular maturation or sex skin phenomenon was evident during the brief intermenstrual period. From that time on, the vaginal smears were composed essentially of basal cells indicating a denuded and immature condition of the mucosa. Menstruation has not occurred again. In both macaques (*monkey 427* and *428*) it would seem that the 5 months and 9 months respectively without conception were not so much periods of true adolescent sterility as times of gonadal inactivity and regression due to poor health.

In contrast to the foregoing, the 4 anovulatory cycles observed for *monkey 426* constituted a true period of sterility. In these cycles characteristic preovulatory smears were observed, the cornification of the superficial epithelial cells often attained 90 to 100%, and follicles approximately 3 mm. in size were palpated. Coitus was permitted when the smears and palpation indicated the probability of ovulation. But the matings were all sterile. The postcoitus smears failed to manifest postovulatory changes; there was no drop in cornification and little of the desquamation and aggregation which imply luteal activity. The cycles of the sterile interval were not only anovulatory but also very irregular in length (52, 29, 36 and 20 days in duration).

The picture for the 3 monkeys which conceived during the second cycle following menarche was much the same. The single preovulatory cycles observed in *monkey 430*, *431* and *432* were likewise characterized by irregularity in length. "Shotty protuberances" but no large follicles were felt on the ovaries. The highly cornified smears of the first half of the cycle failed to give way to smears of less cornified and more folded and aggregated epithelial cells during the last half of the cycle. A prominent sexual skin developed. Nevertheless, it is of great interest that in 3 of the 6 adolescent monkeys this anovulatory interval lasted for only one month.

In every instance the initial ovulation resulted in conception; but only one monkey (*431*) has carried the fetus to term. *Monkey 430* ovulated on the fourteenth day after the onset of the second menstrual flow. A follicle on the left ovary had matured to the size of 4½ mm. The smear showed 85% cornification with much aggregation and folding. Coitus on the day of ovulation resulted in conception, which was evidenced by the growth of a large and persistent corpus luteum with the gradual en-

largement of the left uterine horn. The presence of very large epithelial cells with normal nuclei among many folded, degenerated cells on the smears was also indicative of pregnancy. A scant flow of blood was seen microscopically on the 7th of November, 14 days after conception. Fourteen days postcoitus was slightly early for the placental sign, but it nevertheless approximates the time observed by Hartman (3) for the bleeding of early implantation, i.e. around the time of the next menses, 17 days or so after conception. On the twentieth day of pregnancy a profuse flow of blood began and persisted for 5 days. Just previous to the abortion the corpus luteum receded and the swelling in the left horn of the uterus disappeared. At the onset of pregnancy the weight of *monkey 430* was the same at menarche (3490 gm.), although she had made a month's advance in skeletal development.

On November 28th, the fourteenth day of the second cycle, the vaginal smear of *monkey 432* showed 95% cornification, leucopenia and moderate aggregation and folding. A follicle 3 to 4 mm. in size had been palpated on the right ovary the day before but was not present on the 28th. The animal was mated. The postcoitus smear gave further proof of the presence of the palpable corpus luteum, the partially cornified cells were very clumped, ragged and folded. Fertilization of the expelled ovum had indeed occurred at the time of mating. The placental sign was observed December 15th through the 17th, beginning exactly 17 days after conception. The duration of the pregnancy was short, however, because after 37 days, spontaneous abortion occurred. The month that elapsed between menarche and conception produced no advance in either weight or skeletal development.

After a 4-month sterility interval, *monkey 426* ovulated on the tenth day after the onset of the flow. The smear picture on the ninth day was 90% cornification of desquamated superfiical cells with leucopenia. On the same day, March 3rd, a 5 mm. follicle could be felt on the right ovary. The 5 mm. follicle had disappeared by the morning of March 4th, so the female was immediately placed in the cage with the male. The cornification had decreased slightly and the cells were clumped in abundant secretion on the smear. The uterus became progressively softer and larger. Continued desquamation of large superficial cells with normal round nuclei but keratinized cytoplasm was seen constantly in the smears after conception. The placental sign was microscopically observed from April 4th to 10th, beginning 28 days postcoitus. A diagnosis of pregnancy was made. The pregnancy ended in spontaneous abortion after 82 days. The weight of *monkey 426* at the onset of pregnancy was 3150 gm., which was 350 gm. less than at menarche. She had weighed still less in January; so in March she was showing great improvement in her general condition. The comparative age of this animal had leaped ahead during the sterile interval. At menarche she was rated at 12 yr. 11 mo., while at the onset of pregnancy her skeletal growth was equal to that of the average human female at 13 yr. 6 mo. *Monkey 431*, the one adolescent female to become a mother, was the heaviest, weighing 3900 gm. at menarche and at the time of conception. During her second menstrual cycle, which began October 7th, conception took place. The sex skin exhibited very great swelling and folding of the skin along the peripheral areas of the thighs, buttocks and the upper third of the tail. By the eighth day of the cycle a small follicle was palpable on the anterior end of the right ovary. The follicle matured gradually and on the fourteenth day (October 20th) ovulation occurred. Exposure to the male was permitted the night of the 20th; the ovum was fertilized. At the time of the implantation of the embryo the placental bleeding was evident macroscopically. This leakage of blood from the uterine wall surrounding the placenta began 24 days postcoitus and continued for 3 days. The corpus luteum in the right ovary became very large and re-

mained so. The vaginal smears reflected the constant desquamation of the large partially cornified, folded epithelial cells with many degenerating cells from the thick vaginal mucosa until the end of the second month. The mucosa was completely denuded by the beginning of the third month of pregnancy, only basal or degenerated cells appeared in the smears. Normal parturition took place the night of April 4, 1939 after a 165-day gestation period. A normal, healthy male baby was born. The baby has been thriving, and the mother has been in excellent condition throughout the lactation period.

Thus, 4 ovulations and 4 conceptions following brief periods of sterility have been observed among the 6 adolescent macaques. Three out of the 4 conceptions resulted in spontaneous abortions. Therefore, it would seem that the animals were still too immature to reproduce, although their immaturity was expressed not in failure of ovulation but perhaps in inefficiency of the uterus in maintaining gravidity.

Although the production of maximum cornification in the vaginal smear of ovariectomized rats has been long used as the method for assaying the estrogenic substances (18), a similar correlation has not as yet been established in the monkey. Therefore, it has been impossible to state that high cornification of the epithelial cells of the vaginal mucosa, as seen in the smears, was due to either a high, medium, or low estrone level in the blood of the macaques. Allen (19) produced cornification in the vaginal smears of 5 spayed monkeys by injections of estrogenic hormones. However, neither the degree of cornification nor the amount of hormone required to produce the change in the vaginal mucosa was accurately determined. The experiments of Hisaw, Greep and Fevold (20) on 10 castrated adolescent female macaques treated with estrin-progestin injections also served to prove that a relationship existed between the amount and nature of the ovarian hormone present in the blood and the condition of the vaginal epithelium. By taking biopsies of the vaginal wall Hisaw, et al. (20) found that the epithelium, which was established by the action of estrin alone, decreased when progestin was given in combination with estrin. Using an ovariectomized adult female macaque as the subject, a similar experiment was performed by us except that the vaginal smear technic was substituted for the biopsy method.

The only adult in the group of animals studied was *monkey 433;* its comparative age was estimated as 16+ years. During October and November two excessively profuse menstrual flows were observed. The smears for the intermenstrual period were by no means normal; the leucocytes were too abundant; the epithelial cells appeared ragged and degenerated; and a very profuse secretion of mucus was constantly present. December 12, 1938 a laparotomy[4] was performed; a large omental cyst and both ovaries were removed. The vaginal smears following ovariotomy showed that the vaginal mucosa was swiftly denuded leaving only a thin layer of superficial cells above the basal layer. When it was certain that the denudation of the vaginal mucosa was complete, 3 injections of 2000 R.U. of estradiol benzoate[5] each were administered at 3-day intervals. In this way the vaginal mucosa was stimulated to increased proliferation until maximum cornification of the superficial layer was observed in the smears. The bright red color of the sexual skin areas which had disappeared following castration, returned under the influence of the estradiol injections. Bleeding simulating menstrual flow began 11 days after the last injection. The smears during the 11-day period showed degeneration of the superficial epithelial cells such as char-

[4] We are glad to thank Dr. Sidney Stone for performing the laparotomy.

[5] We are indebted to Dr. Max Gilbert of the Schering Corp. for our supply of estradiol (progynon-B) and progesterone (proluton).

acterized the smears for the anovulatory cycles of the adolescent monkeys. The cornification was again built up to maximum by the 3 injections of 2000 R.U. of estradiol benzoate; then a combined injection of 2000 R.U. of estradiol plus 1 mg. of progesterone was given. The next day the sexual skin was livid and the vaginal smear showed a high degree of aggregation and folding of the much cornified cells. The second day following the double injection there was a slight drop in cornification with clumping of the folded cells in an abundant secretion. Thus, vaginal changes indicative of the ovulatory phase were artificially developed after estradiol-progesterone injections. When the effect of the injections had disappeared, in 12 days time, bleeding much like the normal menstrual flow was observed. This experiment was repeated and identical results were obtained. These results confirm the findings of Hisaw, Greep and Fevold (20) who examined biopsies of the vaginal mucosa after estrin-progestin injections.

The correlations between the vaginal smears and the rectal palpations are summarized in the tables in which each animal showing any follicular development has been included (table 1).

SUMMARY

In order to summarize the observations made on the 7 female monkeys (*Macacus rhesus*), 6 adolescent and 1 adult, the most significant data are presented in table 2.

The data indicate that puberty in the female macaque is followed by gradual physiological development of the genital and endocrine systems until the two are capable of working synchronously and successfully at the function of reproducing. The length of time required to complete this development differs for individual animals but has not been less than a month and seldom more than a year. The interval is a time of anovulatory and irregular menstrual cycles. The mean cycle length for the entire group of 6 adolescent monkeys over the 9-month period averaged 30.6 days with a standard deviation of 11.8. The high incidence of anovulatory cycles is probably due to the failure of the pituitary to produce sufficient luteinizing hormone; follicle stimulating hormone must be present since the growth of the follicles is proved by palpation as well as by the cornification of the epithelial cells in the vaginal smears. The cases of early pregnancy which ended in spontaneous abortion indicate that the adolescent female macaque may be unprepared for reproducing even after the animal is capable of ovulation and conception. The animal which carried a normal fetus to term, having conceived one month after menarche, proves that sterility in the adolescent is a relative phenomenon dependent upon the general health, and the tempo of the growth processes throughout the body for the extent of its duration.

The data show a very high correlation between the findings by rectal palpation and by vaginal smears. The percentage of cornified epithelial cells in the smear provides a rough index of estrone production and therefore of follicular maturation in comparative terms. The massive desquamation of the epithelial cells with aggregation and folding are correlated with ovulation, while degeneration and desquamation are correlated with simple regression of a follicle. The presence of a follicle (on palpation) does not imply that ovulation will follow. If, however, cornification reaches a high level, and progesterone activity is foreshadowed by minimal folding and aggregation of the epithelial cells, then ovulation could be predicted with modest success. It should be noted that conception occurred after mating limited to the ovulation date, which was most accurately determined by combining the observations on sex skin (when present), rectal palpation and the vaginal smears. We believe that employing vaginal smears increases the possibility of accurately timing mating, and that the conceptions occurred at the first ovulation in each of the monkeys.

TABLE I

Date	Vaginal smears			Rectal palpations							Controlled mating		Sexual skin development
				Right Ovary		Left Ovary		Tubes					
	% Cornified epithelial cells	Secretion[1]	Folding[1]	follicles	size, cm.	follicles	size, cm.	Right	Left	Uterus	Exposure to coitus	Conception	
Monkey 426													
1938													
9/29	65	+	0	1 mm.	0.4×0.5 cm.	0	0.8×0.4 cm.	C.[2]	C.	Firm			Edema of central pubic folds.
10/ 3	45	++	+	0	0.5×0.5	0	1×0.5	T.[2]	T.	Relaxed			
Menstruation 10/5–10/7													
10/21	95	+	0	0.5 mm.	0.5×0.5	0.2 mm.	0.4×0.6	T.	T.	Firm			
10/22	95	+	+	2 mm.	0.5×05	0.5 mm.	0.4×0.5	T.	T.	Firm			Edema of central pubic folds.
10/24	95	+	+	2 mm.	0.6×0.5	0	0.4×0.4	T.	T.	Hard			Edema of central pubic folds.
10/25	95	+	+	3 mm.	0.8×0.5	0.1 mm.	0.4×0.3	T.	N.P.	Hard	24 hr.	0	Edema of central pubic folds.
10/26	100	++	+	0	1×0.5	0	0.4×0.3	T.	N.P.	Soft			Edema of central pubic folds.
11/7	90	+++	0	2 mm.	0.8×0.6	0	0.4×0.3	N.P.[4]	N.P.	Firm			
11/8	80	+++	0	0.1 mm.	1×0.8	0	0.5×0.4	N.P.	N.P.	Firm			
11/12	80	+++	0	0	1×0.7	3 mm.	0.5×0.3	N.P.	N.P.	Firm			
11/22	50	+	++	2 mm.	1×0.8	0	0.5×0.4	N.P.	N.P.	Firm			
11/25	40	+++	++++	2 mm.	1×0.8×0.7	0	0.6×0.3	N.P.	N.P.	Firm			Peripheral edema.
11/26	40	+++	++++	3 mm.	1×0.9	0	0.4×0.3	T.	C.	Firm			
Menstruation 11/28–12/1													
12/15	65	+++	+	2 mm.	1×1	0	0.4×0.5	C.	N.P.	Firm			
12/16	60	+++	++	0	1×0.9	0	0.4×0.3	C.	N.P.	Firm			
Menstruation 12/27–12/31													
1939													
1/11	87	++++	0	5 mm.	1×0.4	0	0.4×0.3	C.	N.P.	Firm	24 hr.	0	
1/12	75	++++	+	0	1×0.5	0	0.5×0.4	C.	N.P.	Soft			
Menstruation 2/2–2/6 And again 2/23–2/25													
3/1	65	++	0	2 mm.	1×1	0	0.5×0.3	T.	N.P.	Firm			Perineal area and buttocks livid.
3/3	95	+++	++	5 mm.	1×0.9	0	0.6×0.3	C.	N.P.	Firm			Livid coloration.
3/4	90	++++	+++	0	1×1	0	0.4×0.3	C.	N.P.	Hard	24 hr.	+	Coloration subsiding.
3/6	75	++	++++	0	1×1	0	0.4×0.3	C.	N.P.	Soft		+	
Monkey 428													
1938													
Menstruation 10/3–10/4													
10/20	70	+	+	2 mm.	0.8×0.4	0	0.5×0.2	T.	T.	Soft			
10/21	70	+	+	2 mm.	0.8×0.5	0	0.5×0.1	T.	T.	Firm			
10/22	65	+	++	3 mm.	0.9×0.5	0	0.5×0.1	T.	T.	Firm			
10/24	80	++++	++	0	0.6×0.7	0	0.3×0.2	T.	T.	Soft			Swelling of the central pubic folds.

TABLE 1 (continued)

Date	Vaginal smears			Rectal palpations							Controlled mating		Sexual skin development
				Right Ovary		Left Ovary		Tubes					
	% Cornified epithelial cells	Secretion[1]	Folding[1]	follicles	size, cm.	follicles	size, cm.	Right	Left	Uterus	Exposure to coitus	Conception	
Monkey 428 (contenued)													
10/25	75	++++	++	0.1 mm.	0.6×0.8	0	0.3×0.4	T.	T.	Soft			Increased edema of central folds.
10/26	75	++	++	3 mm.	0.6×0.8	0	0.2×0.3	T.	T.	Soft			Edema of pubic folds increased.
10/27	60	++	+	0.5 mm.	0.6×0.8	0	0.2×0.3	T.	N.P.	Soft			Increased edema.
10/28	60	++	+	1 mm.	0.6×0.8	0	0.2×0.3	T.	N.P.	Firm			Edema.
10/29	65	++	+	0	0.6×0.7	0	0.5×0.5×0.5	T.	N.P.	Firm			Very great edema of pubic folds.
Menstruation 11/16–11/19													
11/21	65	++	0	2 mm.	1×0.6	0	0.5×0.4	N.P.[4]	T.	Firm	24 hr.	□	Peripheral edema and livid color.
11/22	60	+++	+	0	0.6×0.6	0	0.5×0.3	T.	N.P.	Soft			Increased edema and lividity.
11/28	75	++	+	2 mm.	0.8×0.4	0	0.4×0.3	T.	T.	Soft			
11/29	90	+	++	1 mm.	0.9×0.4×0.6	0	0.4×0.3	T.	N.P.	Soft			
11/30	75	++	++	2 mm.	0.8×0.4	0	0.4×0.3×0.5	T.	N.P.	Soft			Sex skin edema increasing.
12/1	65	+++	+	3 mm.	1×0.9×0.5	0	0.3×0.2×0.3	T.	N.P.	Soft	24 hr.	□	Sex skin edema increasing.
12/2	55	+++	+	0	0.8×0.9	0	0.3×0.2×0.3	T.	N.P.	Soft			Sex skin edema and color increasing.
Menstruation 12/29–1/3													
Monkey 430													
1938													
9/29	95	++	0	0	0.6×0.8	3 mm.	1×0.6	N.P.	N.P.	Firm			Edema of central and peripheral areas.
9/30	95	++	0	0	0.6×0.6	0.5 mm.	1×0.4	T.[3]	T.	Soft			Increased edema.
10/3	95	++	0	0	0.6×0.6	1 mm.	1×0.3	N.P.	N.P.	Soft			Increased edema.
10/4	85	++	0	0	0.6×0.7	1 mm.	0.9×0.2	N.P.	T.	Firm			Slight decrease in sex skin.
10/6	75	++	0	0	0.5×0.5	1 mm.	1.5×1	N.P.	T.	Firm			
10/7	70	+	0	0	0.5×0.5	2 mm.	0.4×1	T.	C.	Soft			
10/8	70	+	0	0	0.5×0.5	2 mm.	1×5	T.	C.	Soft			Edema of central and peripheral areas.
10/10	50	+++	0	0	0.6×0.6	2 mm.	1.5×0.5	T.	T.	Soft			Edema decreasing.
Menstruation 10/10–10/17													
10/20	70	++	0	0	0.7×0.4	2 mm.	0.9×0.4	T.	T.	Firm			
10/21	75	++	0	0	0.7×0.4	3 mm.	1×0.4	N.P.	T.	Soft			Livid and edematous in perineal area.
10/22	90	+++	0	0	0.8×0.4	3 mm.	1×0.5	N.P.	T.	Soft			Increased lividity.
10/23	95	+++	+	0	0.8×0.4	4 mm.	1×0.4	N.P.	C.	Soft			Great coloration.
10/24	100	+++	++	0	0.9×0.5	5 mm.	1×0.4	N.P.	C.	Soft	24 hr.	+	Livid.
10/25	85	++	++++	0	0.8×0.4	0	1×0.5	N.P.	C.	Soft		+	Recession of color.
Abortion 11/14–11/17													

[1] + = +25%. [2] T. = Thready. [3] C. = Cordy. [4] N.P. = Not palpable.

TABLE I (continued)

Date	Vaginal smears			Rectal palpations							Controlled mating		Sexual skin development
				Right Ovary		Left Ovary		Tubes		Uterus			
	% Cornified epithelial cells	Secretion[1]	Folding[1]	follicles	size, cm.	follicles	size, cm.	Right	Left		Exposure to coitus	Conception	
Monkey 430 (continued)													
11/29	95	++	+++	0	0.3X0.2	0	1X0.7	N.P.[4]	C.[3]	Soft			Very livid peripheral edema.
Menstruation 12/14–12/16 and 1/6–1/7													
1939													
1/19	75	++++	0	0	0.3X0.2X0.4	0.5 mm.	1X0.6	N.P.	N.P.	Firm			High coloration.
1/20	85	++++	0	0	0.3X0.4	2 mm.	1X0.7	N.P.	N.P.[2]	Firm			Very livid.
1/21	90	++++	+	0	0.3X0.5	2 mm.	1X0.6	N.P.	T.	Firm	48 hr.	0	Very livid.
1/23	90	+++	++	0	0.3X0.4X0.2	0	1X0.6	N.P.	T.	Soft			Very livid.
1/24	85	++	++	0	0.3X0.6	0	1X0.6	N.P.	T.	Firm			Very red.
Menstruation 2/1–2/7													
2/15	75	++	+	0	0.3X0.4	0	1X0.5	N.P.	N.P.	Firm			Fairly red.
2/16	50	++	++	0	0.3X0.6X0.2	0	1X0.4	N.P.	N.P.	Firm			Less livid.
Menstruation 2/22/–2/28													
3/2	85	++	+	0	0.3X0.6X0.3	0.5 mm.	1X0.4	N.P.	N.P.	Firm			Livid.
3/3	80	+++	++	0	0.3X0.4	0	1X0.4	N.P.	N.P.	Firm			Very livid.
3/4	75	+++	++	0	0.3X0.4	0	1X0.5	N.P.	N.P.	Firm			Less livid.
Menstruation 3/13–3/15													
3/17	50	++	0	0	0.2X0.3	1 mm.	1X0.3	N.P.	T.	Soft			
3/18	75	++	+	0	0.2X0.3	2 mm.	1X0.6X0.5	N.P.	C.	Firm			High coloration.
3/20	75	+++	+	0	0.3X0.3	3 mm.	0.8X0.6	N.P.	C.	Firm	24 hr.[5]	0	Livid.
3/21	80	++++	+	0	0.3X0.3	0	0.8X1	N.P.	C.	Hard			Livid.
Monkey 431													
1938													
9/29	85	+++	+	0.2 mm.	1X1	0	0.3X0.4	C.	T.	Soft			
9/30	80	++	+	1 mm.	1.2X0.8	0	0.5X0.6	C.	T.	Soft			
Menstruation 10/6–10/10													
10/15	50	+	0	1 mm.	1.5X1	0	0.6X0.4	N.P.	N.P.	Firm			Extensive peripheral edema.
10/17	65	++	0	1 mm.	1.5X1	0	0.5X0.4	N.P.	N.P.	Soft			Peripheral edema.
10/18	75	+++	0	4 mm.	1.5X1	0	0.5X0.6X.4	N.P.	N.P.	Soft			Peripheral edema.
10/19	95	+++	+	4 mm.	1.5X1	0	0.5X0.4	C.	N.P.	Soft			Great folding and swelling.
10/20	95	++++	+++	5 mm.	2X1	0	0.5X0.6	C.	N.P.	Soft	24 hr.	+	Extensive peripheral edema.
10/21	85	++	++++	0	2X1X1	0	0.5X0.7X0.4	C.	N.P.	Firm		+	Peripheral edema.

TABLE 1 (continued)

Date	Vaginal smears			Rectal palpations							Controlled mating		Sexual skin development
				Right Ovary		Left Ovary		Tubes		Uterus			
	% Cornified epithelial cells	Secretion[1]	Folding[1]	follicles	size, cm.	follicles	size, cm.	Right	Left		Exposure to coitus	Conception	
Monkey 432													
1938													
9/29	85	++	0	0	1×0.5	1 mm.	0.8×0.4	T.	T.	Firm			
9/30	75	+	0	2 mm.	1×0.2	0	0.8×0.8	C.	T.	Soft			
Menstruation 10/3–10.4													
10/11	65	++	0	2 mm.	1×0.4	0	0.6×0.6	T.	N.P.	Soft			
10/12	65	+	0	3 mm.	0.8×0.4	0	0.6×0.5	T.	N.P.	Soft			
10/13	60	+	0	3 mm.	0.8×0.4	0	0.6×0.5	C.	T.	Soft			
10/14	50	+	+	3 mm.	0.7×0.4	0	0.6×0.5	T.	T.	Firm			
10/15	50	++	+	0	0.7×0.4	0	0.7×0.6	T.	T.	Firm			
10/17	85	++	0	0	0.8×0.4	3 mm.	0.9×0.5	T.	T.	Firm			
10/18	95	++	+	0	0.5×0.7	0	1×1.5	T.	C.	Soft	24 hr.	0	
10/19	75	++	++	0	0.5×0.2	0	0.6×0.2	T.	C.	Soft			
11/3	40	++	0	0	0.5×0.5×0.5	0	0.6×0.4	N.P.	T.	Soft			Edema of central pubic folds.
Menstruation 11/15–11/17													
11/22	90	++	0	3 mm.	1×0.6	0	0.4×0.4×0.4	N.P.	N.P.	Soft	24 hr.	0	Edema of central pubic folds.
11/25	75	++	+	2 mm.	1×0.6	0	0.3×0.3	C.	N.P.	Soft			Peripheral edema increasing.
11/26	85	++	+	3 mm.	1×0.5×0.8	0	0.3×0.2	C.	T.	Soft			Peripheral edema great.
11/28	95	+++	++	0	1×0.4	0	0.3×0.4	C.	T.	Soft	24 hr.	+	Lividity and great peripheral edema.
11/29	75	+++	++	0	0.8×0.4	0	0.3×0.4	T.	N.P.	Soft		+	Great lividity and edema.
Abortion 1/4–1/11													

[1] + = +25%. [2] T. = Thready. [3] C. = Cordy. [4] N.P. = Not palpable

TABLE 2

No. of monkey	426	427	428	430
Sexual maturity	Adolescent	Young adolescent	Adolescent	Advanced adolescent
No. of cycles observed	4	0	3	6
Average length of cycle	34.5 days	0 days	38.6 days	25 days
Range of cycle length	52–20 days	0 days	52–20 days	34–18 days
Observation of menarche	October, 1938	January, 1939	October, 1938	September, 1938
Weight at menarche	3500 gm.	2950 gm.	3290 gm.	3490 gm.
No. of ovulatory cycles	1	0	0	1
Day of ovulation[1]	10th	—	—	14th
Relation of first ovulation to menarche	In 5th cycle	—	—	In 2nd cycle
No. of anovulatory cycles	4	0	3	6
No. of exposures to male[2]	3	0	2	3
Conceptions	+	—	—	+
Weight at onset of pregnancy	3150 gm.	—	—	3490 gm.
Duration of pregnancy	82 days[3]	—	—	20 days[3]
Sexual skin development	1. Edema of central pubic folds. 2. Edema of peripheral folds. 3. Lividity of perineal area.	None	1. Edema of central pubic folds. 2. Edema of peripheral folds.	1. Edema of central pubic folds. 2. Peripheral edema. 3. Lividity of perineal area.

No. of monkey	431	432	433
Sexual maturity	Advanced adolescent	Advanced adolescent	Adult
No. of cycles observed	0	3	1
Average length of cycle	0 days	28.6 days	28 days
Range of cycle length	0 days	42–40 days	0 days
Observation of menarche	September, 1938	October, 1938	—
Weight at menarche	3800 gm.	3400 gm.	—
No. of ovulatory cycles	1	1	0
Day of ovulation[1]	14th	14th	—
Relation of first ovulation to menarche	In 2nd cycle	In 2nd cycle	—
No. of anovulatory cycles	0	3	1
No. of exposures to male[2]	1	3	0
Conceptions	+	+	—
Weight at onset of pregnancy	3800 gm.	3400 gm.	—
Duration of pregnancy	165 days	37 days	
Sexual skin development	2. Peripheral edema. 3. Lividity of perineal areas.	1. Edema of central pubic folds. 2. Peripheral edema. 3. Lividity of perineal areas.	3. Lividity of perineal area, nipples and face.

[1] Calculated from the onset of the menstrual cycle.
[2] Exposure to male whenever ovulation was anticipated. Ovulation did not occur except once in each case.
[3] Spontaneous abortion.

Therefore, from the observations made on 6 adolescent female monkeys (*M. rhesus*) over a 9-month period the following conclusions are drawn.

The vaginal smear technic in correlation with rectal palpation and controlled mating has distinct value as a method for determining the time of ovulation.

Menarche in the female macaque occurs at an average weight of 3400 gm. The menstrual cycle of the adolescent female macaque, is very irregular in length (30.6 days $\pm$ 11.8).

Anovulatory cycles which characterize the adolescent period of macaques are probably due to insufficient luteinization of the developed follicle.

The sterile period of the adolescent monkey may be but one month, or it may continue for a year or more depending on the health and growth rate of the animal.

Spontaneous abortion occurs very readily in adolescent monkeys which conceive at the time of their initial ovulation.

The authors are glad to acknowledge the helpful interest of the late Dr. T. Wingate Todd, the editorial advice of Dr. T. T. Zuck, director of the Brush Foundation, and of Dr. Wm. H. Weir, professor of gynecology, Western Reserve University.

REFERENCES:

1. ASHLEY-MONTAGU, M. F.: *Quart. Rev. Biol.* 14: 13; 192. 1939.
2. HARTMAN, C. G.: *Science* 74: 226. 1931.
3. HARTMAN, C. G.: *Contrib. Embryol.* 433: 1. 1932.

4. Zuckerman, S.: *Proc. Zool. Soc. London* 691. 1930.
5. Zuckerman, S.: *Eugenics Rev.* 28: 37. 1936.
6. Yerkes, R. M.: *Science* 81: 542. 1935.
7. Elder, J. H.: *Yale J. Biol. & Med.* 10: 347. 1938.
8. Papanicolaou, G. N.: *Am. J. Anat.* 52: 519. 1933.
9. Davis, E. M., and C. G. Hartman: *J.A.M.A.* 104: 279. 1935.
10. Zuckerman, S., G. Van Wagenen and R. H. Gardiner: *Proc. Zool. Soc. London* 108: 385. 1938.
11. Todd, T. W.: Atlas of Skeletal Maturation. Part I. Hand. C. V. Mosby Co. 1937.
12. Hartman, C. G.: *Am. J. Obst. & Gynec.* 24: 600. 1933.
13. Burr, H. S., R. T. Hill and E. Allen: *Proc. Soc. Exper. Biol. & Med.* 23: 109. 1935.
14. Burr, H. S., L. K. Musselman, D. Barton and N. B. Kelley: *Yale J. Biol. & Med.* 10: 155. 1937.
15. Zuckerman, S.: *Proc. Zool. Soc. London* 139. 1932.
16. Engle, E. T., and M. C. Shelesnyak: *Human Biol.* 6: 431. 1934.
17. Boaz, F.: *Human Biol.* 4: 307. 1932.
18. Allen, E., and E. A. Doisy: *J.A.M.A.* 81: 819. 1923.
19. Allen, E.: *Contrib. Embryol.* 19: 1. 1927.
20. Hisaw, F. L., R. O. Greep and H. L. Fevold: *Am. J. Anat.* 61: 483. 1937.

INFLUENCE OF THE UTERUS ON THE CORPUS LUTEUM

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IT IS GENERALLY recognized that the persistence of the corpus luteum is a determining factor in the normal sex cycle and in the initiation of normal pregnancy. The difference in the length of persistence of the corpus luteum in normal pseudopregnant and pregnant animals respectively, indicates that it does not have a predetermined or inherently fixed life span. This must mean that the continuation of corpus luteum activity and hence, of the normal sex cycle and of pregnancy, depends upon extra-luteal factors. The present work is concerned with one of these.

On a priori grounds the cessation of luteal activity may be due to the withdrawal of a supportive factor, or to the appearance of an inhibitory factor, or to both. In regard to the first possibility, it is known that hypophysectomy terminates early pregnancy in all species of animals in which this has been done, presumably by withdrawing the gonadotropic hormone of the anterior hypophysis (1). This is supported by the fact that pregnancy can be maintained after hypophysectomy in the rabbit, with suitable injections of gonadotropic hormone or of progestin (2, 3). That this relationship between the anterior pituitary and the corpus luteum is not confined to pregnancy, is shown by the fact that the functional corpora of pseudopregnant rats also lose their luteal activity following hypophysectomy (4). It would appear from this evidence that the anterior hypophysis is the source of a supportive influence, the withdrawal of which may be partly or wholly responsible for regression of the corpus luteum.

There is also evidence for the existence of a uterine activity which, either directly or through the pituitary gland, inhibits the corpus luteum. Loeb has shown that total hysterectomy following estrus in adult guinea pigs prevents luteal regression and permits persistence of the corpora for 60 to 100 days (5, 6). In the rabbit, a non-cyclic animal, hysterectomy following the appearance of the functional corpus luteum of pseudopregnancy leads to prolongation of the life span of the corpus luteum (7, 8, 9). Hysterectomy following estrus in the rat does not lead to a persistent corpus luteum (6, 10, 11). However, Bradbury has recently reported that hysterectomy of the pseudopregnant or pregnant rat does result in persistent corpora (12). The common factor in all of this work on the different animals appears to be the removal of the uterus in the presence of functional corpus luteum. However, this may not be the only factor since hysterectomy in the opossum (13) or monkey (14) has not been followed by luteal persistence.

It is significant that the corpora of hysterectomized guinea pigs, rabbits and rats persist for a time interval approximately equivalent to their normal gestation period. Thus in guinea pigs where the period of pregnancy is about 65 days, hysterectomy causes persistence of corpora for 60 to 100 days (6); in rabbits where the pregnancy period is about 32 days, the corpora persist for 25 to 29 days (7); in rats where the period of gestation is about 21 days, the corpora are maintained for about the same length of time after hysterectomy (12). These data indicate that the uterus contains a corpus luteum antagonist, the removal of which allows luteal persistence. They suggest that the maintenance of the corpus luteum during pregnancy, may depend upon the removal or neutralization of the antagonist in the uterus.

[1] Aided by the A. B. Kuppenheimer Fund.

In view of the above, and also because of the degenerative changes in the ovaries which have been reported to follow hysterectomy in the human female (15), the further investigation of the possible endocrine rôle of the uterus seemed of interest and importance.

METHODS AND RESULTS

These experiments were begun before the report of Bradbury referred to above. Our first step, was therefore similar to his work and yielded the same results. (*a*) Hysterectomy performed during any stage of the sex cycle in the rat did not produce luteal persistence. (*b*) Hysterectomy of pregnant rats resulted in maintenance of

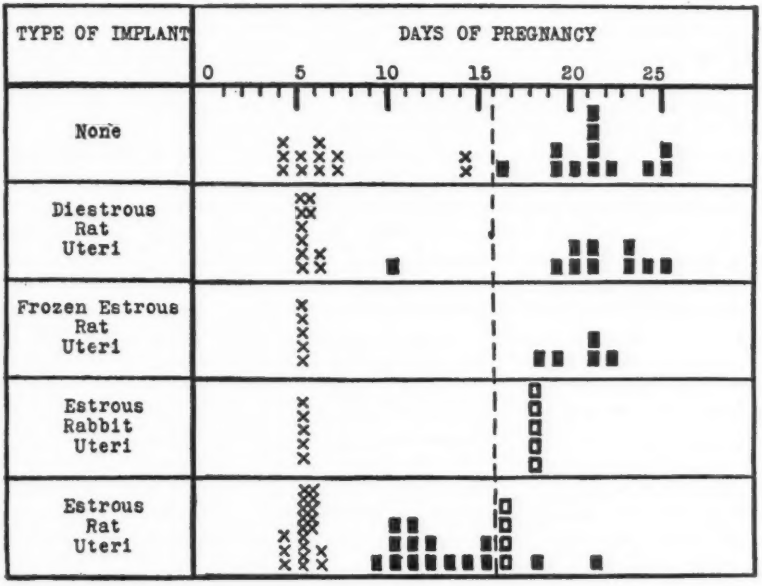

Fig. 1. The effect of different implants on the life span of the corpus luteum in hysterectomized pregnant rats. Crosses indicate the days of pregnancy on which hysterectomy was performed. Implantations, when made, were at the same time. Black squares indicate the days of the first appearance of vaginal estrus, indicating regression of the corpus luteum. Hollow squares indicate killing of the animal for postmortem examination before the appearance of vaginal estrus. The vertical broken line marks the earliest day of corpus luteum regression in the non-implanted animals.

their corpora. Since functional corpora inhibit ovulation and vaginal estrus, the appearance of a fully cornified smear would indicate cessation of corpus luteum activity. Thus the appearance of a fully cornified estrous smear was taken to represent the time of regression of function of the corpora. The results of these experiments are shown in figure 1. It will be noted that the animals on the average went into estrus at about what would have been the 21st day of pregnancy. This reaction appears to be independent of the stage of pregnancy at which the hysterectomy was performed. There was only one exception, in which case the corpora persisted only until the 16th day of pregnancy.

Having confirmed the fact that hysterectomy of pregnant rats results in maintained luteal function, we attempted to inhibit the corpora of such animals by uterine

implantation. In another group of pregnant rats, at the time of hysterectomy, estrous or diestrous uteri were implanted. In each case one-half of the uterus was slit open, and then both pieces were cut up and implanted intraperitoneally. The results of these experiments are found in figure 1. It will be seen that 13 of the 19 rats which received estrous uterine implants went into estrus before the 16th day, most of them on or about the 11th day. The other 6 experiments were negative. Implantation of diestrous uteri, on the other hand, produced no regressive effect in 9 of 10 rats, only one positive effect being observed. Postmortem gross examination showed that the implants of both estrous and diestrous uteri had apparently "taken." In general, there was no correlation between the gross appearance of the implant and the results obtained.

In order to determine whether or not our positive results with estrous uterine implants depended on whether or not a "take" had occurred, estrous uteri were used in such a manner as to minimize the possibility of viable uterine tissue being implanted. For this purpose, rat estrous uteri which had been frozen with dry ice and rabbit estrous uteri were implanted into hysterectomized pregnant rats. Gross postmortem examination of these animals revealed that in no case did these uteri "take." The results of these experiments are shown in figure 1. It will be seen that estrous uteri employed in such fashion have no effect on the corpora of the experimental animal. These experiments conclusively demonstrate that the estrous uterus must "take" to be effective in its antagonistic action on the corpus luteum.

Histologic examination of the implanted uteri, both estrous and diestrous, revealed marked degenerative, necrotic and inflammatory changes. The epithelium of the endometrium was involuted maximally, the endometrial stroma moderately, and the muscularis minimally. There were no significant differences in this respect between the various groups. In about half the cases there was an obliteration of the uterine architecture which could not be related to the various groups of implants. The significant histological differences between the implants which gave positive results and those which yielded negative findings were the presence of an apparently greater amount of viable endometrial stroma with a lesser degree of degenerative changes, and an increased number of endometrial glands in the former group as compared to the latter. Presumably, therefore, the factor which causes luteal regression is related to the glands, to the endometrial stroma or to both.

DISCUSSION

Our results clearly show that implants of estrous uteri can inhibit functional corpus luteum, and that removal of this antagonistic influence in the presence of functional corpora allows extra persistence of luteal function in the rat.

Since the corpora of pregnancy ordinarily persist in the presence of the gravid uterus, some other factor must operate to negate the uterine antagonist. This factor, whose end result is luteal maintenance, may reside in either the fetus or in the placenta. The finding that fetal destruction has no influence on the corpora of pregnancy, as evidenced by placental retention (16, 17), makes it very probable that the placenta is responsible for the removal of the uterine antagonist. This viewpoint is further supported by the findings that (*a*) following removal of both placenta and fetus, the corpora regress within 2 to 3 days (16) and (*b*) a corpus luteum stimulating substance has been extracted from rat placentae (4).

The question arises as to the nature of this uterine corpus luteum antagonist. Although at first thought, this activity appears to be hormonal, the reaction may be dependent on the metabolic activity of the uterus. It has been recently shown that

the non-involuted uterus converts estrone to estriol (18), and that estrone stimulates the corpus luteum in rabbits (19). Bradbury (20) has suggested that the effects of hysterectomy might be ascribed to the constant stimulation of the corpora by estrone which has accumulated due to interference with its normal metabolism by the removal of the uterus. Our experiments with uterine implantations are easily explained on this hypothesis by assuming that the implanted uterus restores the normal metabolism of estrone and hence leads to corpus luteum regression. On the other hand, our results are equally well explained on a hormonal basis. According to this view, the estrous uterus would contain a hormone which inhibits the corpus luteum. In pregnancy, it might be supposed that the uterine hormone is antagonized by the placenta with resulting luteal persistence throughout gestation. The concept of uterine hormones which participate in the sex cycle is not a new one (cf. 21). However on the basis of our present work, it is impossible to determine whether the uterine antagonism to the corpus luteum is hormonal or metabolic.

SUMMARY

The uterus and placenta have important influences on the life span of the corpus luteum. The manner in which these influences may be exerted is briefly discussed.

REFERENCES

1. ALLEN, E.: Sex and Internal Secretions. 2nd Edition: Williams and Wilkins, Baltimore, 1939 (p. 931).
2. ROBSON, J. M.: *J. Physiol.* 86: 415. 1936.
3. ROBSON, J. M.: *Edinburgh Med. J.* 43: 395. 1936.
4. ASTWOOD, E. B., AND R. O. GREEP: *Proc. Soc. Exper. Biol. & Med.* 38: 713. 1938.
5. LOEB, L.: *Am. J. Physiol.* 83: 202. 1927.
6. LOEB, L.: *Proc. Soc. Exper. Biol. & Med.* 20: 441. 1923.
7. ASDELL, S. A., AND J. HAMMOND: *Am. J. Physiol.* 103: 300. 1933.
8. LOEB, L., AND M. G. SMITH: *Am. J. Anat.* 58: 1. 1936.
9. GILLARD, J. L.: *Am. J. Physiol.* 120: 300. 1937.
10. LONG, J. A., AND H. M. EVANS: *Mem. Univ. Calif.* 6: 1. 1922.
11. MURPHY, D. P.: *Anat. Rec.* 60: 77. 1934.
12. BRADBURY, J. T.: *Anat. Rec. suppl.* 70: 51. 1937.
13. HARTMAN, C. G.: *Am. J. Anat.* 35: 25. 1925.
14. BURFORD, T. H., AND A. W. DIDDLE: *Surg., Gynec. & Obst.* 62: 701. 1936.
15. MARX, R., H. R. CATCHPOLE AND B. S. MCKENNON: *Surg., Gynec. & Obst.* 63: 170. 1936.
16. NEWTON, W. H.: *J. Physiol.* 84: 196. 1935.
17. KIRSCH, R. E.: *Am. J. Physiol.* 122: 86. 1938.
18. PINCUS, G.: *Cold Spring Harbor Symp. Quant. Biol.* 5: 44. 1937.
19. ROBSON, J. M.: *J. Physiol.* 90: 1937.
20. BRADBURY, J. T.: In discussion of G. Pincus (18).
21. REYNOLDS, S. R. M.: Physiology of the Uterus: Hoeber, New York and London, 1939 (p. 281 ff).

THE RESPONSE OF RAT UTERI TO HORMONE INJECTIONS[1]

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SEVERAL INVESTIGATIONS have recently been reported dealing with changes in respiration of the reproductive tract in rodents. The oxygen consumption or glycolysis of uteri of the rat or mouse throughout the estrous cycle, in castrates and after the injection of various glandular extracts and crystalline hormones has been studied (1–8). The results are somewhat contradictory. On the whole, the majority of investigators seem of the opinion that some increase in respiration results at some stages of the estrous cycle, and also after injection of female hormones. Aschheim and Gesemius (2) reported as well a respiratory response of the seminal vesicles of immature mice to injection of male hormone. Moreover heightened respiration presumably manifests itself in male and female sex organs subsequent to injection of hormones, and prior to ensuing growth, at least in the uterus.

That the metabolism of various organs of the rat is influenced by the several stages of the estrous cycle has been amply brought out by the extensive work of Victor and coworkers, (9–13). The tissues studied were thyroid, pituitary, liver and kidney. An extensive study of the aerobic and anaerobic glycolysis of the rat uterus has been reported by Kerly (14).

Astwood (15) has recently demonstrated that injections of hormone into immature female rats produce an increase in uterine weight which is roughly proportional to the amounts of hormone injected. The increase in dry weight is relatively slight, indicating that the bulk of weight gained by the immature uterus is due to swelling or absorption of water.

In connection with experiments undertaken for the purpose of studying changes in the oxidase system and concentrations of reducing substances in the rabbit uterus with the onset of pregnancy or subsequent to injection of hormone (16), it was thought advisable to test the metabolic response of rat uteri to hormone injection. The situation presents sufficiently different aspects to justify the effort, even though for purposes of extraction of cytochrome oxidase or other enzyme systems. The rat material is obviously at a disadvantage as compared with the rabbit. The fact that the growth of the rat uterus occurs within 6 hours after injection indicates immediate action of the hormone on some physiological activities of the uterus. Whatever these changes may be it is of interest to know whether they affect the respiration and metabolism of the whole tissue.

EXPERIMENTAL

Our first series of experiments was performed with female rats 30 days old. Varying amounts of estrone or estriol dissolved in olive oil were injected intraperitoneally, the animals killed 6 hours later and the uteri removed and weighed. They were then placed in Barcroft-Warburg respirometer vessels for the purpose of

[1] Aided by grants from Rockefeller Foundation and the National Research Council Committee for Problems of Sex. Works Progress Administration Project No. 665-14-3-726.

TABLE 1. CHANGES IN WET WEIGHT OF UTERI IN NORMAL AND INJECTED RATS, 30 DAYS OLD

Date of experiment	Treatment	Wet wt. immediately after excision	Wet wt. after 3 hr. in M/15 phosphate buffer	Difference	Dry weight	Dry wt. / Wet wt.
	Uninjected controls	mg.	mg.	mg.	mg.	%
		31.6	36.3	+ 4.7	5.6	17.8
		27.9	31.8	+ 3.9	5.7	20.1
		18.8	20.8	+ 2.0	2.9	15.5
		17.7	21.6	+ 3.9	3.1	17.6
		22.2	22.2	+ 0.0	4.6	20.8
		22.5	24.7	+ 2.2	4.7	20.9
		20.6	21.7	+ 1.1	3.5	16.9
		28.0	33.1	+ 5.1	4.3	15.2
		23.6	24.9	+ 1.3	3.7	15.7
		17.0	21.0	+ 4.0	2.8	16.2
		20.6	26.4	+ 5.8	3.0	14.6
		19.4	24.0	+ 5.0	3.0	15.3
		19.5	26.7	+ 7.2	3.0	15.4
		20.0	25.3	+ 5.3	3.2	15.8
		17.0	22.9	+ 5.9	2.7	15.8
4/13/39	0.05γ estriol	40.8	35.0	− 5.8	4.6	11.3
		32.8	25.8	− 7.0	3.5	10.7
		39.5	33.9	− 5.6	4.6	11.5
		39.7	35.0	− 4.7	4.6	11.6
4/13/39	1.2γ estriol	33.5	29.9	− 3.6	3.6	10.7
		35.8	32.2	− 3.6	3.8	10.5
		55.1	49.0	− 6.1	5.6	10.0
		37.3	36.1	− 1.2	4.2	11.2
4/14/39	2.0γ estriol	50.5	36.4	−14.2	7.1	13.9
		40.3	34.3	− 6.0	4.2	10.4
		45.3	40.1	− 5.2	5.0	11.1
4/14/39	3.0γ estriol	26.5	27.0	+ 0.5	3.2	11.9
		46.4	37.3	− 9.1	4.8	10.2
		42.2	42.0	− 0.2	4.3	10.2
		36.2	34.2	− 2.0	4.5	12.5
4/12/39	1.0γ estriol	31.0	26.4	− 4.6	3.1	10.0
		28.9	24.5	− 4.4	3.0	10.4
		46.2	35.4	−10.8	4.2	9.1
		37.0	31.6	− 5.6	3.4	9.1
4/12/39	Urinary estrone					
	Inactive	22.5	20.2	− 1.3	2.5	11.1
		24.5	23.0	− 1.5	4.2	17.2
	Active	38.0	26.6[1]	−11.4	2.7	7.1
		22.7	17.4[1]	− 5.3	2.7	12.0
4/12/39	Urinary estriol					
	Inactive	17.0	15.6[1]	− 1.4	2.9	17.3
		24.0	22.9[1]	− 1.1	4.8	20.0
	Active	38.4	28.5[1]	− 9.9	5.0	13.0
		31.0	24.0[1]	− 7.0	2.9	9.5
4/17/39	Active	30.7	23.2	− 7.5	3.0	10.0
		35.8	24.5	−11.3	3.4	9.4
		31.8	25.1	− 6.7	3.4	10.9

[1] These uteri kept in Ringer-Locke solution.

measuring rates of oxygen uptake of respective uteri. The animals used were either untreated, or injected with varying amounts of crystalline estriol or estrone or urinary estrogens derived from normal and cancerous patients (16).

It was observed that the rat uteri undergo a change in wet weight after being placed in the buffer solution. This can be noted in presumably isotonic M/15 phosphate buffer pH 7.3. The results are given in table 1.

The rat uteri were dried by gentle pressure between filter papers or paper towels and weighed on a Roller-Smith precision balance. These weights are referred to as 'wet weights immediately after excision.' The uteri were then placed for varying intervals in buffer solutions in vials or in respirometer vessels. At the end of various intervals they were recovered and weighed to determine loss or gain in weight.

It can be seen from table 1 that uteri of uninjected and hormone injected rats

TABLE 2

Injected estrogen	Time killed	Wt. at time of excision	Wt. after 3 hr. in buffer	Difference	Dry weight	Dry wt. / Wt. wt.
		mg.	mg.	mg.	mg.	%
Horse urine estrogens	1 hr. after injection	19.1	27.7	+ 8.6	3.2	16.8
		13.4	20.2	+ 6.8	2.2	16.5
		22.4	29.7	+ 7.3	3.3	14.7
	2 hr. after injection	20.4	24.4	+ 4.0	3.0	15.0
		28.2	32.8	+ 4.6	4.1	14.5
		23.0	25.2	+ 2.2	3.2	13.9
	6 hr. after injection	33.5	23.8	− 9.7	3.2	9.5
		32.5	23.3	− 9.2	3.4	10.4
		36.1	27.3	− 8.8	3.8	10.3
No injection	Control rats	19.9	28.1	+ 8.2	3.0	15.1
		20.8	31.5	+10.7	3.4	16.1
		7.7	12.9	+ 5.2	1.1	14.4

behaved differently in identical media. Uteri of untreated animals or of animals injected with oil alone showed a gain in wet weight, which apparently means that they absorbed water from the environment while uteri of hormone injected rats showed a loss. These changes were not only apparent in isotonic M/15 phosphate buffer but also in serum, Ringer-Locke and 0.15 M phosphate solutions.

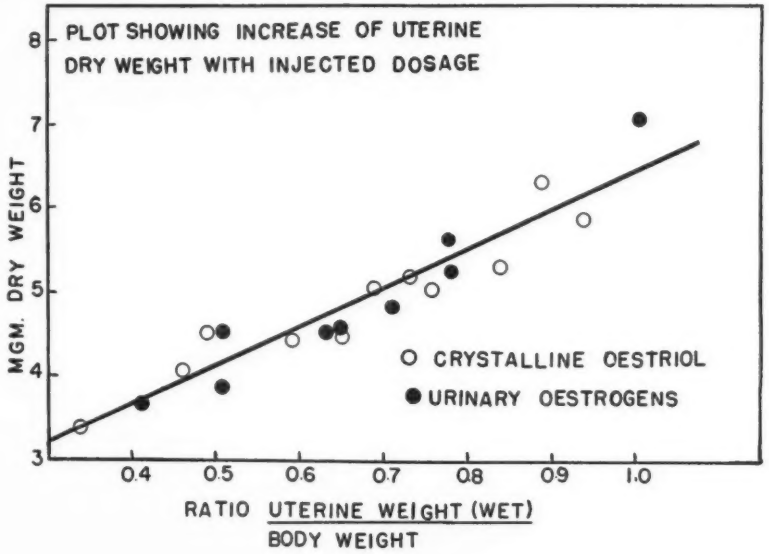
In a recent report Holden (17) presented evidence for the occurrence of vasodilatation in immature rats subsequent to estrogen injection. He also called attention to the fact that "vasodilatation following a single injection of 0.1γ estradiol closely parallels the early increase in water content of the uterus." Yet it is not likely that the vasodilatation can account for the swelling of uteri from untreated animals or the shrinkage of stimulated ones. Assuming that loss of blood occurs in the latter it could not be as high as the shrinkage values indicate.

The respiration was measured in terms of dry weight of uteri. After the respiration measurements were taken, the uteri were removed from the vessels, dipped rapidly in distilled water to wash off adhering buffer, placed in hollows of porcelain plates used for pH determinations in an oven at 106–110°C. overnight, and weighed. In determining the ratios of dry weight over wet, the original wet weight after excision was employed.

The values given in table 1 are taken from a random series of successive experi-

ments in which the entire range of different hormone quantities were injected. The control values are of the uninjected rats used as controls each time.

It should be noted that the rats injected with estrogens derived from urines of patients showed irregularity with regard to swelling, hence dry over wet weight ratios. These samples were bioassayed by a modified Astwood method (unpublished) and not all were active, as indicated in table 1. Those injections which had no hormone activity do show in two cited cases a slight loss in wet weight after submergence in buffer, characteristic of hormone-affected uteri. This never occurred with uninjected or oil injected controls and it is likely that the urine extract though free from estrogens may have still contained traces of substances affecting permeability.



This period intervening between injection and the usual time of killing animals were also studied with regard to changes in weight and respiration of uteri. The data presented in table 2 indicate that uteri excised from injected animals one or three hours after injection of estrogen underwent swelling, but when excised 6 hours after injection showed the usual tendency to shrink. The tendency to swell, which makes them resemble unstimulated uteri, was highest in uteri removed one hour after excision. Moreover, the amount of swelling was the same as in unstimulated uteri. Uteri removed after 3 hours showed diminished swelling, while uteri excised after 6 hours showed the usual behavior characteristic of the stimulated condition with regard to dry weight and shrinkage.

The ratios of dry to wet weight express the amount of swelling taking place subsequent to injection (15). This ratio seemed to vary roughly with amounts of injected hormone although the maximum effect was often obtained from the smallest injection.

A diminution of this ratio with increased injection would be obtained if only swellings occurred and the absolute dry weight remained constant. Actually the dry weight did tend to increase. Figure 1 shows the increase in the absolute uterine dry

weight with injected dosage by plotting the dry weight against the ratio of uterine wet weight to body weight. This ratio is a function of the quantity of hormone injected and is better suited for the relationship represented because it corrects for the body weight of the test animals.

To obtain reliable respiration rates it is important to check the method of obtaining dry weights on a control series of animals as well as the effect of the medium in which the uterine tissues are kept during the experiment. Sterile rabbit serum and phosphate buffer gave similar results with regard to percentage dry weight as well

TABLE 3. OXYGEN UPTAKE PER MG. DRY WEIGHT PER HOUR OF UTERI FROM 30-DAY-OLD RATS. PHOSPHATE BUFFER M/15, PH=7.4; T=35°C.

Date	Treated	cu. mm. O_2 per mg. dry weight per hour
1/19/39	normal	4.47
	2.0γ estriol	4.81
1/23/39	normal	4.28
	1.5γ estriol	4.90
1/25/39	normal	4.50
	6.0γ stilbestrol	5.30
2/1/39	normal	5.40
	2.0γ estriol	5.20
2/6/39	normal	4.00
	2.0γ estriol	5.20
2/7/39	normal	5.20
	1.0γ estriol	5.30
3/3/39	normal	4.40
	2.0γ estriol	5.30
2/27/39	normal	4.70
	2.0γ estriol	5.80
3/25/39	normal	3.72
	0.1γ estriol	4.60
	0.5γ estriol	3.66
	1.5γ estriol	4.70
	3.0γ estriol	4.39

as respiration rates. Thus, the percentage dry weight of uteri kept in buffer was 17.4%; in one-third buffer and two-thirds serum or in serum alone the percentages were 19.0 and 19.6% respectively.

The rate of oxygen uptake of rat uteri was measured with a single uterus per respirometer vessel or with several properly identified. In each case straight lines, when O_2 uptake is plotted against time, were obtained at least during the first 90 minutes of the experiment. The results are expressed in terms of c.mm. of oxygen per hour per mg. dry weight of tissue. The data are given in table 3.

The data do not seem to permit a definite conclusion with regard to the estrogen effect on respiration. Slight increases in the respiration of uteri derived from stimulated animals may be noted on the basis of the figures presented in table 3. But it may be questioned whether these differences are significant because of the overlapping of the individual values of which they are averages. Moreover, as will be seen from table 5 below, the respiration values of stimulated uteri of 22-day-old rats definitely showed no increased rates.

A similar series of experiments was performed with 22-day-old rats. Table 4a gives the wet weight changes in uninjected and olive oil injected control animals. The injections with pure olive oil were made for the purpose of noting whether the observed changes in wet weight could possibly be due to the oil rather than the hormone. The results indicate that oil is not responsible for the wet weight changes. The data of table 4a indicate as well that a highly hypertonic buffer solution produced the usual increase in wet weight of untreated uteri. This must be taken to mean that the salt concentration of the external medium is not the only regulatory mechanism of the phenomena of swelling or loss of water.

TABLE 4a. CHANGES IN WET WEIGHT OF UTERI OF UNINJECTED AND OIL INJECTED FEMALE RATS 22 DAYS OLD (UTERI KEPT 3 HR. IN 0.15M PHOSPHATE BUFFER PH 7.4)

Weight immediately after excision	After 3 hr. in 0.15M phosphate buffer pH 7.4	Difference	Dry weight	Dry wt. / Wet wt.
		Uninjected		
mg.	mg.	mg.	mg.	%
17.9	27.4	+9.5	3.2	17.7
20.0	29.4	+9.4	3.5	17.5
21.3	30.7	+9.4	3.7	17.2
20.2	29.2	+9.0	3.6	17.8
19.8	28.1	+8.3	3.5	17.4
19.4	22.8	+3.4	3.0	15.2
19.9	23.8	+3.9	3.1	15.3
19.4	24.2	+4.8	3.0	15.2
20.1	24.0	+3.9	3.2	15.5
		Oil injected		
24.4	34.1	+9.7	4.7	19.1
28.0	35.8	+7.8	4.8	17.1
22.5	29.2	+6.7	3.8	16.9
25.1	34.9	+9.8	4.4	17.5
26.7	32.7	+6.0	4.3	15.9
23.9	30.1	+7.2	4.1	17.2
25.3	32.8	+7.5	4.9	19.3
19.0	27.2	+8.2	3.4	17.6
20.5	28.6	+8.1	3.7	17.8
21.4	29.5	+8.1	3.7	17.1
18.2	20.9	+2.7	2.5	13.8
20.8	25.6	+4.8	3.7	17.8
20.5	25.4	+4.9	3.3	15.9
18.4	22.5	+3.1	2.8	14.9

Table 4b shows the correlation of changes in wet weight in excised uteri with amounts of injected hormone. It should be noted that uteri of 22-day-old rats injected with 0.05γ or 0.10γ estriol behaved like controls in that they were swollen in M/15 phosphate buffer, while uteri of rats injected with higher estriol concentrations invariably showed loss of water in the same medium. This variation with amount of hormone injected was always found in the 22-day-old rats and often in 30-day-old ones. In the latter group, however, uteri of rats injected with low concentrations of hormone behaved most of the time in the same manner as those injected with higher quantities.

The oxygen uptake of uterine tissues of normal and injected 22-day-old rats was studied in the way previously described. The data are given in table 5. It will be noted that no significant differences can be detected between respiration rates of uteri derived from uninjected, oil injected and hormone injected animals.

TABLE 4b. CHANGES IN WET WEIGHT OF UTERI OF UNINJECTED AND INJECTED 22-DAY-OLD FEMALE RATS

Control					Hormone Injected				
Immediately after excision	After 3 hr. in buffer M/15	Difference	Dry wt.	Dry wt. / Wet wt.	Immediately after excision	After 3 hr. in buffer M/15	Difference	Dry wt.	Dry wt. / Wt. wt.
5/31/39	mg.	mg.	mg.	%		mg.	mg.	mg.	%
13.9	14.7	+0.8	2.3	15.6	0.05 γ estriol				
16.6	16.5	−0.1	2.8	17.0	29.5	31.8	+ 2.3	4.6	15.6
14.7	13.4	−1.3	2.3	15.3	23.5	25.2	+ 1.7	3.4	14.5
6/6/39					0.25γ				
21.2	28.0	+7.3	2.9	13.8	31.5	33.8	+ 2.3	4.6	14.5
20.5	27.2	+6.7	2.9	14.2	27.6	27.8	+ 0.2	3.6	12.9
6/8/39					0.5γ				
28.8	32.2	+3.4	3.3	11.3	28.6	24.3	− 4.3	3.2	11.2
27.3	33.4	+6.1	4.4	16.0	31.2	28.0	− 3.2	3.5	11.0
20.8	23.6	+2.8	3.2	15.5					
					3.0γ				
					30.1	25.4	− 4.7	3.0	10.0
					30.3	27.0	− 3.3	3.3	10.8
					6/8/39				
					0.05γ				
					32.6	34.8	+ 2.2	3.8	11.6
					24.7	25.4	+ 0.7	2.8	11.1
					0.1γ				
					17.0	19.1	+ 2.1	1.9	11.2
					15.7	18.5	+ 2.8	2.4	15.0
					16.7	20.3	+ 3.6	2.7	15.9
					0.25γ				
					36.5	31.6	− 4.9	4.4	12.1
					40.7	36.8	− 3.9	4.0	10.0
					24.9	23.0	− 1.9	3.0	12.1
					0.5γ				
					47.5	32.9	−14.6	5.6	11.7
					30.3	25.0	− 5.3	3.7	12.0
					33.6	26.6	− 7.0	3.6	10.5
					1.0γ				
					42.8	35.0	− 7.8	4.4	10.1
					28.0	24.2	− 3.8	3.1	11.0
					42.2	34.8	− 7.4	4.7	11.0

A study has also been made of the influence of some known inhibiting or accelerating substances on uterine respiration. The data are presented in table 6. All uteri tested came from 30-day-old animals. It will be seen that only iodoacetamide in the concentrations used and 0.1M NaCN completely inhibited the respiration, while glyceraldehyde and urethane inhibited only about 50% of the normal respiratory rate.

DISCUSSION AND SUMMARY

The data here presented indicate that during a period of 6 hours after injection no large changes in respiration based upon dry weight take place in uteri of rats injected with estrogenic hormones. While these hormones produced an increase in wet weight due to absorption of water as demonstrated by Astwood, the respiration seemed to be normal.

TABLE 5. RESPIRATION OF UTERINE TISSUE OF 22-DAY-OLD RATS

Date	Treatment	O_2 in cu. mm. per mg. per hour	Date	Treatment	O_2 in cu. mm. per mg. per hour
5/31/39	Normal	5.45	6/10/39	normal	5.00
	0.1γ estriol	5.70		0.1γ	4.95
	0.25γ	5.83		0.25γ	5.40
	0.5γ	4.90		0.5γ	5.05
	1.0γ	5.45		1.0γ	5.27
6/6/39	Normal	6.20	8/10/39	normal, oil injected	4.70
	0.05γ	5.15			5.00
	0.05γ	5.50		normal, no oil	5.00
	0.1γ	5.90			4.80
	1.0γ	6.08			
6/7/39	0.05γ	4.40			
	0.25γ	4.42			
	0.5γ	4.95			
	3.0γ	4.78			
				32 days old	
6/8/39	Control	5.50	5/26/39	control	5.20
	2.0γ estriol (one hour after injection)	5.20		horse estrogen one hr. after injection	4.60
	2.0γ estriol (3.25 hr. after injection)	5.70		3 hr. after injection	4.85
	0.05γ	5.20		6 hr. after injection	6.05
	0.25γ	4.60			5.15
	0.5γ	4.60		human urinary estrogen	5.00
	1.0γ	4.75			5.40
					5.28

TABLE 6. EFFECT OF VARIOUS SUBSTRATES ON RESPIRATION OF UTERI OF 30-DAY-OLD RATS

Date	Substance added	Treatment	O_2 Uptake in cu. mm. per mg. dry weight per hour
5/26/39	4 mg. glyceraldehyde	injected urine estriol	4.70 before addition 2.54 after
5/19/39	2 mg. iodoacetamide	inj. (horse estrog.)	0.0
	0.5 cc. cytochrome	injected	5.2 after addition
	16 mg. Na succinate	injected	4.00
	Controls		4.02
	Controls		3.75
5/12/39	Control	injected (estriol)	3.60
	8 mg. succinate	injected (estriol)	4.90
	0.5 cc. cytochrome	injected (estriol)	4.10
5/11/39	Control	injected (estriol)	4.10
	1.0 cc. 0.01M NaCN	injected (estriol)	2.70
	8 mg. Na succinate	injected (estriol)	6.20
	2 mg. iodoacetamide	injected (estriol)	0.00
	0.5 cc. cytochrome	injected (estriol)	4.40
4/18/39	Control	urine estrogens	4.15
	2 mg. urethane	urine estrogens	2.85
	2 mg. Na succinate	urine estrogens	3.40
4/17/39	Control	injected (estriol)	4.50
	1 cc. 4.9% glucose	injected (estriol)	4.45
	1 cc. 0.1M NaCN	injected (estriol)	0.60

The swelling itself presents peculiarities in that the stimulated, excised uteri lost weight in presumably isotonic solutions, while the uteri of uninjected animals gained weight even in highly hypertonic media. This problem clearly requires further study. There is, however, a definite increase in dry weights of uteri of hormone injected animals killed 6 hours after injection. This increase is proportional to the dosage of hormone injected, but is not as large as the increase in wet weight. As a consequence the percentage of dry weights of affected uteri was smaller than that of uninjected ones.

The fact that iodoacetamide, glyceraldehyde and cyanide produced inhibition of respiration suggests that the oxygen uptake of the uterus is dependent upon glycolytic and aerobic mechanisms. That cytochrome does not enhance respiration is not surprising since it is not likely to penetrate the cells.

REFERENCES

1. David, J. C.: *J. Pharm. Therap. Med.* 43: 1. 1931.
2. Aschheim, S., and H. Gesemius: *Arch. Gynäk.* 153: 434. 1933.
3. Kochmann, M., and W. Wagner: *Ztschr. ges. Exper. Med.* 53: 705. 1926.
4. Khayyal, M. A., and C. M. Scott: *Quart. J. Exper. Physiol.* 24: 249. 1934.
5. Khayyal, M. A., and C. M. Scott: *Quart. J. Exper. Physiol.* 25: 77. 1935.
6. Kaufman, C., C. Muller and C. Muhlbock: *Klin. Wchnschr.* 11: 14. 1932.
7. Muhlbock, O., and C. Kaufmann: *Arch. Gynäk.* 149: 623. 1932.
8. Bungeler, W., and K. Ehrhardt: *Klin Wchnschr.* 10: 593. 1931.
9. Victor, J., and D. H. Andersen: *Am. J. Physiol.* 115: 130. 1936.
10. Victor, J., and D. H. Andersen: *Am. J. Physiol.* 120: 154. 1937.
11. Victor, J., and D. H. Andersen: *Am. J. Physiol.* 122: 296. 1938.
12. Victor, J., D. H. Andersen and M. R. Prest: *Am. J. Physiol.* 115: 121. 1936.
13. Andersen, D. H., M. R. Prest and J. Victor: *Am. J. Physiol.* 110: 445. 1937.
14. Kerly, M.: *Biochem. J.* 31: 1544. 1937.
15. Astwood, E. B.: *Endocrinology* 23: 25. 1938.
16. Graubard, M., and G. Pincus: *Am. J. Physiol.* in press.
17. Holden, R. B.: *Endocrinology* 25: 293. 1939.

THE EFFECT OF STILBESTROL ON THE BASAL METABOLISM OF EXPERIMENTAL HYPERTHYROID RATS

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DODDS, GOLBERG, LAWSON AND ROBINSON (1) investigated possible estrogenic agents bearing a close structural resemblance to estrone capable of ready synthesis. They suggested the term stilbestrol for the most potent substance of the synthetic compounds. This was fully active in ovariectomized rats in a dosage between 0.3 and 0.4 γ. These authors (2) later reported that diethylstilbestrol was fully estrogenic in doses of 0.004 mg. given subcutaneously dissolved in oil, and 0.001 mg. given by mouth. This would indicate that the substance is several times more potent than estrone and at least as potent as estradiol. Dodds, Golberg, Lawson and Robinson (3) reported on the estrogenic activity of esters of diethyl stilbestrol. It was found that esterification reduced the activity but that the effect was prolonged.

Sherwood (4) reported that 1000 mg. of desiccated thyroid per kg. of body weight given daily for 3 days to thyroidectomized rats affected the basal metabolism for a period of 24 to 28 days. The administration of amniotin immediately following the thyroid feeding brought about a return to the original basal metabolic level within a period of 12 days.

In view of the above findings, it seemed desirable to determine the effect of stilbestrol, a synthetic estrogen, upon the basal metabolism of experimental hyperthyroid animals.

EXPERIMENTAL PROCEDURE

The animals used in this investigation were obtained from the Wistar Institute and placed in a constant temperature room (28°C.) 2 months prior to the basal metabolism determinations. The above precautions seemed necessary since the environment was considerably changed from that of their previous existence. It was possible to use litter mates as controls against the experimental animals by means of 8 determinations within a period of several hours.

Sixteen rats were ovariectomized and thyroidectomized after the normal basal metabolism had been established. The metabolic rate was determined at 28° C. and the data calculated in calories per square meter of body surface according to the formula of Lee (5).

When the hypothryoid level had been determined, desiccated thyroid was administered to each animal for a period of 3 days, the daily dose being 1000 mg. per kg. of body weight. Metabolism tests were then run daily until the hypothyroid level had again been established.

Nine of the above animals were given 3 daily injections of 10 mg. of stilbestrol immediately following the cessation of thyroid feeding.

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Ten ovariectomized and thyroidectomized animals were used as controls for basal metabolic conditions throughout the period of experimentation.

RESULTS

Table 1 presents the data which are typical of the findings in the present investigation. The weight, percentage decrease in metabolism due to thyroidectomy, percentage increase in basal metabolism due to thyroid therapy and the number of days necessary for the animals to return to the hypothyroid level are given.

TABLE 1. EFFECT OF STILBESTROL ON THE BASAL METABOLISM OF EXPERIMENTAL HYPERTHYROID RATS

Animal	Weight gm.	% decrease in B.M.R. due to thyroidectomy	% increase in B.M.R. due to thyroid therapy	Return to hypothyroid level, days
		Thyroid controls		
1	225	28.3	77.8	28
2	230	27.4	70.1	22
3	245	16.0	58.0	26
4	240	22.6	96.2	24
5	200	39.9	80.5	26
6	195	19.0	73.4	27
7	215	25.2	68.1	25
Average	221	25.5	74.9	25
		Experimentals		
8	185	17.4	94.7	18
9	210	18.0	55.9	15
10	185	22.6	70.7	10
11	200	25.2	72.2	13
12	215	19.2	75.0	16
13	230	23.4	81.4	10
14	235	21.0	76.0	12
15	190	18.3	70.8	15
16	215	22.0	85.0	18
Average	207	20.8	75.7	14

Thyroidectomy brought about a decrease from an average normal of 32.9 to 24.7 calories per square meter of body surface per hour. Thyroid feeding raised the basal metabolism from the hypothyroid level of 24.7 to 43.2 calories per square meter per hour.

The thyroid controls show a decrease of approximately 25% following thyroidectomy and an increase of approximately 75% following thyroid feeding. The animals returned to the hypothyroid basal metabolic level in from 22 to 28 days.

The stilbestrol treated animals show a decrease of approximately 21% following thyroidectomy and an increase of approximately 76% following thyroid feeding. These animals returned to the hypothyroid basal metabolic level in from 10 to 18 days.

DISCUSSION

The present investigation indicates that stilbestrol does affect the basal metabolism of experimental hyperthyroid rats in the same manner as amniotin as reported by Sherwood (4). No attempt was made to use smaller doses of stilbestrol than 5 mg. or larger than 20 mg. No difference in the results was observed by varying the dosage within this range. It is possible that a much smaller dose would have been just as effective. No toxic results were observed by the administration of the large doses which is in accord with the findings of Dodds (6).

Dodds, Lawson and Noble (7) report that diethyl stilbestrol has been found to have an action similar to estrone on the uterus of ovariectomized rats, on the mating reaction, vagina and uterus of immature rats, on the uterus of immature rabbits, and on the feathers of capons. By vaginal-smear assay on ovariectomized rats under the conditions described, the synthetic substance was approximately two and one-half times as active as estrone. Nipple growth, similar to that caused by estrone, was produced in the guinea pig. These same authors report that preliminary observations indicate that the synthetic substance has appreciably less stimulating action on the mammary tissue than estrone.

The data of the present investigation indicate that stilbestrol is not as effective as amniotin in the reduction of basal metabolism. It is possible that this might have been due to the amount used in the experimentation.

SUMMARY

The hypothyroid basal metabolic level was obtained in approximately one-half the time when stilbestrol injections were given to experimental hyperthyroid rats immediately following the cessation of thyroid feeding. Stilbestrol was apparently not as effective as amniotin in the reduction of basal metabolism.

The author wishes to express thanks to Dr. J. A. Morrell of E. R. Squibb & Sons for the generous supply of stilbestrol and to Dr. E. L. Sevringhaus for his helpful criticism of the manuscript.

REFERENCES

1. Dodds, E. C., L. Goldberg, W. Lawson and R. Robinson: *Nature* 141: 247. 1938.
2. Dodds, E. C., L. Goldberg, W. Lawson and Robinson: *Nature* 142: 34. 1938.
3. Dodds, E. C., L. Goldberg, W. Lawson and R. Robinson: *Nature* 142: 211. 1938.
4. Sherwood, T. C.: *Am. J. Physiol.* 124: 114. 1938.
5. Lee, M. O.: *Am. J. Physiol.* 89: 24. 1929.
6. Dodds, E. C.: *Practitioner* 142: 309. 1939.
7. Dodds, E. C., W. Lawson and R. L. Noble: *Lancet* 1: 1389. 1938.

THE METABOLISM OF THE THYROTROPHIC AND GONADOTROPHIC HORMONES

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MANY INVESTIGATORS have sought to demonstrate the presence of thyrotrophic hormone in the serum or the urine of patients suffering from thyroid disorders; the reports, which have recently been summarized by Jones (1), are conflicting, though there is some agreement that thyrotrophic hormone is excreted for a time after thyroidectomy, in animals or in man. Smith and Moore (2), and Krogh and Okkels (3), injected large amounts of the hormone into normal guinea pigs and were unable to recover demonstrable quantities from the urine. Loeser (4), however, observed some renal excretion of injected thyrotrophic hormone in a normal and a thyroidectomized rabbit. He also showed that the injected hormone disappeared from the blood of a normal rabbit in about an hour, while it was still demonstrable after 4 hours in the blood of a thyroidectomized rabbit; this difference, he suggested, could either be explained by supposing that the thyroid itself played an important part in removing thyrotrophic hormone from circulation, or might be attributed simply to the depressed rate of urine flow in the thyroidectomized animal.

Urinary excretion of gonadotrophic hormones, especially those of chorionic origin has of course been demonstrated many times. Lipschütz, Fuente-Alba and Vivaldi (5) indicated that injected "prolan" disappeared from the circulation of nephrectomized rabbits, but unusually slowly and at a rate comparable to the rate of disappearance (6) of the gonadotrophic principle of pregnant mare serum, to which the kidney is impermeable. It seems certain that gonadotrophic hormones, even those which are readily excreted, may undergo destruction or inactivation within the body; and it is often supposed that the gonads themselves are active in permanently removing gonadotrophic hormones from the blood. Several investigators have explained along these lines the finding that a given dose of gonadotrophic hormone may have an abnormally vigorous effect upon the single ovary of a unilaterally-ovariectomized animal. In such experiments, however, the part played by the animal's own hypophysis has not been controlled although it appears not to be negligible, according to recent unpublished studies at this university on unilaterally-ovariectomized hypophysectomized rats. Moreover, the rate of disappearance of gonadotrophic hormone of pregnant mare's serum from the blood of rabbits injected with this preparation was not decreased by castration (6).

The aim of this investigation was to discover how far the thyroid and the gonads are responsible for the destruction or inactivation of the specific trophic hormones which act upon them. The experiments are of two main types. In the first group, large doses of a thyrotrophic preparation were injected into normal and into thyroidectomized animals, and the amounts of hormone recoverable from the urine were estimated by biological assay. Similarly, a gonadotrophic extract of anterior hypophysis was injected into normal and castrate male and female guinea pigs, whose urines were assayed. In the second group of experiments, potent thyrotrophic extracts were

brought into intimate contact with macerated thyroid tissue, which was subsequently removed by filtration, the amount of thyrotrophic activity remaining in the filtrates being determined biologically. Similar experiments were performed with gonadotrophic preparations and ovarian tissue. Macerated muscle tissue and liver tissue were used as controls of the specificity of the observed inactivations.

Five different hormone preparations were used in these experiments, of which 4 were prepared in this laboratory. The thyrotrophic fraction *127* was prepared from ox pituitaries; total solids, 0.82%. Thyrotrophic fraction *63* was prepared from hog pituitaries; total solids, 1.1%. One-hundredth cc. b.i.d. of each of these was an effective dose, tested on the thyroids of immature guinea pigs. Both of these extracts represented an isoelectric protein fraction recently described by Collip (7).

Gonadotrophic fraction *113* (total solids, 0.36%) represented an alcohol precipitate from a primary dilute acid extract of hog glands. Gonadotrophic fraction *25* was likewise prepared from hog pituitaries; total solids, 1.2%. It represented the isofiltrate from a protein fraction like *127*. Three injections daily, for 3 days, each of 0.0133 cc., constituted an effective dose for immature female rats.

A commercial preparation from the serum of pregnant mares, "gonadin" (Cutter Laboratories) was used in one experiment. Each cc. is stated to contain 200 Cole-Saunders units.

In the first group of experiments, the thyrotrophic preparation *127* was injected subcutaneously into adult guinea pigs weighing 350 to 550 gm., in doses usually of 3 cc. each, thrice daily for 5 days (except in a few instances where the animals failed to survive). The urines were, in most cases, pooled for each sub-group, though collected separately and daily. The urines were injected subcutaneously, in doses of 2 cc. or less, twice daily for 5 days into immature guinea pigs weighing 160 to 220 gm. On the sixth day, these test animals were killed, the thyroids removed, weighed and fixed in Heidenhain's 'Susa' mixture for histological study. Control urines were obtained from the same adult guinea pigs before commencing the series of injections and were similarly assayed. The thyroidectomized animals were operated on only 2 to 4 days before beginning the injections, in the hope of forestalling any overproduction of thyrotrophic hormone by the hypophysis as a possible consequence of thyroidectomy. In the later experiments of this group, special attention was paid to the possibility of leakage of the injected thyrotrophic solution from the site of injection and of consequent contamination of the urines with active material. Accordingly, the practice of pooling the urines was abandoned and special precautions were taken, including making the injections in shaved areas with long, thin needles, and sealing the punctures with collodion before returning the animals to the metabolism cages.

The results of these experiments are summarized in table 1. The thyroid weights of the test animals furnish little information, though it may be observed that the urines of the injected animals stimulated the development of thyroids weighing 40 mg. or more in 6 out of 13 test animals, while glands of this size were not seen in the 6 animals injected with control urines. Application of the Chi-square test with Yates's correction shows that such a distribution might arise by chance alone, though the probability is small ($P = 0.075$). The records of thyroid hyperplasia, on the other hand, suggest very strongly that thyrotrophic hormone was present in the urines of the thyroidectomized injected animals in much larger amounts than in the urines of the injected intact animals or in the control urines. This would imply either that the thyroid gland itself does play a considerable part in the destruction or inactivation of the thyrotrophic hormone, or, less plausibly, that the metabolic rates had already decreased sufficiently to retard destruction of the hormone in other tissues. Delayed excretion is less likely to be of importance in these experiments, where the urines were

collected for several days. It is rather unfortunate that these conclusions should have to be based solely on the interpretation of the histological preparations. The decisions on the presence and degree of hyperplasia were in most cases confirmed by a second observer, but it is to be acknowledged that they were not made in ignorance of the plan of the experiment or of the weights of the thyroids examined. It may be observed that the negative results with the urines of the intact injected animals indicate that the risk of accidental contamination with active material is slight.

Experiments were also performed upon one normal and one thyroidectomized rabbit, injected intravenously with 3 cc. of extract *127* thrice daily for 6 days. In this

TABLE 1. THYROID WEIGHT AND HYPERPLASIA IN YOUNG GUINEA PIGS INJECTED WITH URINE OF NORMAL AND THYROIDECTOMIZED GUINEA PIGS TREATED WITH THYROTROPHIC EXTRACT *127*

Donor guinea pigs supplying urine	Dose extract *127* administered to donors thrice daily	Dose of urine administered to indicator guinea pigs twice daily for 5 da.	Thyroid weights of indicators	Thyroid hyperplasia
		cc.	*mg.*	
4 intact animals	3 cc. for 4 to 5 days	2	43	0
		1	33	0
		0.5	39	0
		0.2	27	0
4 thyroidectomized animals	3 cc. for 4 to 5 days	2	38	++
		1	40	++
		0.5	42	++
		0.2	42	+
1 thyroidectomized	3 cc. for 5 days.	2	37	+++
	3 cc. for 5 days	2	28	+
	3 cc. for 2 days	2	43	++++
	1.5 cc. for 5 days	2	41	+++
	0.5 cc. for 5 days	2	32	0
2 thyroidectomized	Not treated	2	35	0
		1	33	0
1 thyroidectomized	Not treated	2	23	++
1 thyroidectomized	Not treated	2	23	++
2 intact animals	Not treated	2	37	0
		1	36	0

case the urines themselves gave no clear evidence of thyrotrophic activity when tested as before, but a preparation from the urine of the thyroidectomized rabbit, made by acetone precipitation as described by Jones (1) displayed definite activity, the thyroids of the guinea pigs receiving the larger doses showing increased weight and definite hyperplasia. This confirms Loeser's view (4) that the rabbit kidney is not impermeable to thyrotrophic hormone.

Experiments similar in plan were carried out with the pituitary gonadotrophic extract *113*, injected in doses of 2 cc. twice daily for 3½ days into adult guinea pigs, caged in pairs: a pair of intact males injected, a pair of castrate males injected, a pair of castrate male controls, and 3 similar pairs of females, i.e. castrates and normals injected, and castrate controls. Every care was taken to avoid contamination of the urines with active material. The assays were performed upon immature female rats, weighing 35 to 52 gm. when killed, the urine from each pair of guinea pigs being injected thrice daily for 3 days, subcutaneously, in various doses; on the fourth day the hymen was examined, vaginal smear preparations inspected for signs of estrus, the animals killed and their ovaries weighed. The results are summarized in table 2.

TABLE 2. OVARIAN WEIGHTS, IN MG., OF IMMATURE FEMALE RATS INJECTED WITH THE URINE OF NORMAL AND CASTRATE GUINEA PIGS TREATED WITH GONADOTROPHIC EXTRACT *113*

Source of urine	Vol. of urine collected	Dose of urine, cc., given thrice daily for three days			
		1.0	0.5	0.25	0.1
	cc.	mg.	mg.	mg.	mg.
Castrate females, injected	76	39[1]	34[1]	36[1]	27
Castrate males, injected	84	39[1]	27[1]	21[1]	18
Intact females, injected	34[2]	22	20	16	16
Intact males, injected	113	21	16	18	20
Castrate females, controls	138	13	20	18	17
Castrate males, controls	134	14	20	17	15

[1] Vaginal patency was observed.
[2] Urine collection incomplete.

Ovaries weighing more than 25 mg. were observed in 6 out of 8 tests on urine of in-injected castrate animals, and in none of the other tests; vaginal patency was similarly distributed, and was usually accompanied by vaginal estrus. The Chi-square test shows that the odds are 100 to 1 against the theory that chance alone has determined the appearance of large ovaries in the rats treated with the urine of the injected castrates and not elsewhere. It may therefore be affirmed that pituitary gonadotrophic hormone can be recovered from the urine of male and female castrate guinea pigs into which it has been injected, while it is present in much smaller quantities, if at all, in the urine of intact male and female guinea pigs similarly treated. This strongly suggests that inactivation of gonadotrophic hormone takes place, to a considerable extent, in the gonads. It is, of course, possible that castration led to endogenous production of gonadotrophic hormone in quantities not directly determinable but capable of synergistically enhancing the activity, and thus apparently increasing the recovery of the hormone administered.

TABLE 3. EFFECT OF CONTACT WITH SUSPENSION OF THYROID TISSUE ON POTENCY OF THYROTROPHIC EXTRACTS, TESTED ON YOUNG GUINEA PIGS

Dose administered twice daily for 5 days	Thyroid wt., mg.	Thyroid hyperplasia
Thyrotrophic extract *63* in contact with thyroid tissue		
0.5	38	0
0.5	33	0
0.5	40	0
Thyrotrophic extract *63* and thyroid extract administered separately		
0.5	53	++
0.5	50	+
0.5	48	+
Thyrotrophic extract *127* in contact with thyroid tissue, buffered		
2.0	47	+++
1.0	43	++
1.0	35	+
0.5	32	0
Thyrotrophic extract *127* in contact with muscle tissue, buffered		
1.0	40	+++
1.0	56	++++
0.5	40	++++
Thyrotrophic extract *127*, in buffered saline		
0.5	52	+++

TABLE 4. EFFECT OF CONTACT WITH SUSPENSION OF OVARIAN TISSUE ON POTENCY OF GONADOTROPHIC EXTRACT 25, TESTED ON IMMATURE FEMALE RATS

Dose given thrice daily for 3 days, cc.	Wt. of ovaries, mg.	Presence of estrus	Vagina patency
Gonadotrophic extract exposed to contact with ovarian tissue			
0.5	63	+	+
0.3	50	+	+
0.2	34	?	o
0.1	18	o	o
Gonadotrophic extract and extract of ovarian tissue administered separately			
0.5	65	+	+
0.3	65	+	+
0.2	72	+	+
0.1	50	+	+

The first in vitro experiments on inactivation of thyrotrophic hormone with thyroid tissue were carried out with the hog pituitary extract 63. Ten cc. of this extract were added to 80 cc. of saline and 10 cc. of fresh macerated beef thyroid gland; the mixture was stirred for 15 minutes at 20°C., and 15 minutes at 37°C., and centrifuged. The supernatant liquid was filtered and 10 cc. of 95% alcohol added; it was then tested on young guinea pigs, in doses of 0.5 cc. twice daily for 5 days; thyroid weights and hyperplasia were recorded as before. Control guinea pigs were injected separately with thyrotrophic extract (diluted to the equivalent volume with saline and alcohol), and with the filtrate from a saline suspension of macerated beef thyroid, so that the two sets of guinea pigs presumably received parallel amounts of the same materials, except insofar as thyrotrophic hormone may have been inactivated by contact with the thyroid suspension. If, as many authors quoted by Loeser (4) affirm, thyroid extracts inhibit the response to thyrotrophic hormone even when separately injected, this inhibition should be constant in both sets of test animals. Moreover, experiments arranged to investigate this problem suggested that inhibition of this type should not be an important factor in the circumstances selected here. The results of these experiments are recorded in the upper portion of table 3. It is clear that the thyroids of the guinea pigs which received thyrotrophic hormone and thyroid extract separately were heavier, and showed more hyperplasia, than those of the guinea pigs treated with the thyrotrophic extract which had been in contact with thyroid tissue. The difference in thyroid weights between these two first groups of table 3 was tested by 'Student's' method (8) using the statistic t, and was found highly significant ($P = 0.007$). It may therefore be concluded that hog thyrotrophic hormone

TABLE 5. EFFECT OF CONTACT WITH OVARIAN TISSUE AND MUSCLE TISSUE ON POTENCY OF GONADOTROPHIC EXTRACT 25, TESTED ON IMMATURE FEMALE RATS

Dose administered thrice daily for 3 days	Ovarian wt., mg.	Presence of estrus	Vagina patency
Gonadotrophic extract in contact with ovarian tissue			
1	64	+	+
0.5	20	o	o
0.25	26	o	o
Gonadotrophic extract in contact with muscular tissue			
1	85	+	+
0.5	36	?	+
0.25	39	o	+
Gonadotrophic extract and ovarian extract administered separately			
0.5	59	+	+
0.25	33	+	+

is inactivated or otherwise removed from solution by contact with a suspension of beef thyroid gland tissue.

This finding, of course, does not establish the specificity of the interaction; further experiments were accordingly undertaken, and are reported in the lower part of table 3. These were similar in plan to those discussed above, save that a bovine thyrotrophic hormone, 127, was used, and a saline-buffer mixture at PH 8 was employed to control possible effects of PH upon the stability or solubility of the thyrotrophic hormone; in this case, too, tests were made of the effect of exposing the hormone to a buffered saline suspension of macerated beef muscle. In this case, the thyroid weights of the test animals provided no information; the hyperplasia records, however, do very definitely suggest that muscle has less effect than thyroid gland in inactivating or taking-up thyrotrophic hormone, the difference being most marked

TABLE 6. EFFECT OF CONTACT WITH OVARIAN TISSUE AND LIVER TISSUE ON POTENCY OF MARE'S SERUM GONADOTROPHIC EXTRACT, TESTED ON IMMATURE FEMALE RATS

Dose, single injection	Ovarian wt., mg.	Presence of estrus	Vagina patency
Gonadin in contact with ovarian tissue			
1	30	+	+
0.5	20	?	o
0.25	17	o	o
Gonadin and ovarian extract administered separately			
1	34	+	+
0.5	29	+	+
0.25	20	o	o
Gonadin in contact with liver tissue			
1	41	+	+
0.5	28	o	+
0.25	19	o	o

with the smallest dose, as might be anticipated. Nevertheless, in view of the uncertainty of the method of assay and the restricted number of tests, a specific action of thyroid gland tissue on thyrotrophic hormone cannot be regarded as established beyond doubt.

Similar experiments were performed with the gonadotrophic extract of hog pituitaries, 25, brought into contact with macerated hog ovarian tissue in saline suspension under conditions entirely comparable with those described above for the thyrotrophic extract, save that in this case the final dilution of the extract was 5 to 1 instead of 10 to 1. In the first series, which is not reported, the dosage selected was apparently too high, so that maximal responses were obtained in every case. Better results were obtained with lower doses, and are reported in table 4. It will be seen that the ovaries of the immature rats treated with ovarian extract and gonadotrophic hormone separately are larger, at each dose level, than those of the rats treated with parallel amounts of gonadotrophic hormone exposed to contact with ovarian suspension. The differences in ovarian weights, tested by the statistic t, approach statistical significance ($P = 0.05$) closely, though they do not attain it. The records of vaginal estrus and opening of the hymen also indicate, without completely establishing, removal of gonadotrophic hormone from solution by ovarian tissue. In this experiment, the solutions to be tested biologically had been kept for a considerable time in the refrigerator.

A further, similar test was accordingly made with fresh solutions prepared in the same way, and is reported in table 5. Here the effect of exposure to macerated hog muscle was investigated for comparison. It is evident that the gonadotrophic hormone

survived contact with muscle tissue better than with ovarian tissue; the difference between the two series of ovarian weights is statistically significant, tested by t, and is confirmed by the records of vaginal patency. It seems not unlikely that the muscle tissue did remove a proportion of the gonadotrophic hormone from solution, but it was clearly less effective than ovarian tissue.

Finally, the effect of contact with macerated hog ovary and with macerated liver upon the mares' serum preparation 'gonadin' was investigated; the contact in this case was for 30 minutes at room temperature (23°C.), while in the previous experiment it was continued for one hour, with continuous stirring in both cases. The results with gonadin, recorded in table 6, are not decisive: liver was apparently less destructive than ovarian tissue, but the difference is not quite statistically significant in this case.

SUMMARY

The urine of thyroidectomized adult guinea pigs injected with thyrotrophic hormone apparently produced thyroid hyperplasia in young guinea pigs, when the urine of similarly-treated intact adult guinea pigs, and of untreated guinea pigs whether intact or thyroidectomized, usually failed to do so. Small amounts of thyrotrophic hormone have been recovered from the urine of a thyroidectomized rabbit treated with this hormone.

The urine of castrate male and female guinea pigs injected with hypophyseal gonadotrophic hormone produced ovarian enlargement, opening of the vagina, and usually estrus in immature female rats; while the urine of similarly treated intact male and female guinea pigs, and of untreated castrate guinea pigs, failed to do so.

Thyrotrophic hormone was partly removed from solution when brought into contact with a saline suspension of thyroid tissue. It is probable that muscle tissue was less active in this respect.

Gonadotrophic hormone was partly removed from solution when brought into contact with a saline suspension of ovarian tissue. Muscle suspensions had less activity, if any; it is probable that liver suspensions were also less active.

These experiments are considered to suggest that the thyroid gland plays a considerable part in permanently withdrawing thyrotrophic hormone from the blood, and that the gonads likewise withdraw gonadotrophic hormone from the blood. The experiments with gonadotrophic hormone yielded more decisive results, both in vivo and in vitro, than those with thyrotrophic hormone.

I am greatly indebted to Dr. J. B. Collip for extending to me the hospitality of his laboratory, and for much valuable advice; to Dr. H. Selye, for examining histological preparations; and to Dr. D. L. Thomson for assistance in the preparation of this manuscript.

REFERENCES

1. Jones, M. S.: *Endocrinology* 24: 665. 1939.
2. Smith, M. G., and E. Moore: *Proc. Soc. Exper. Biol. & Med.* 30: 735. 1933.
3. Krogh, M., and H. Okkels: *Compt. rend. Soc. de Biol.* 113: 635. 1933.
4. Loeser, A.: *Arch. exper. Path. u. Pharmakol.* 176: 697. 1934.
5. Lipschütz, A., A. Fuente-Alba and T. Vivaldi: *Compt. rend. Soc. de Biol.* 120: 323. 1935.
6. Catchpole, H. R., H. H. Cole and P. B. Pearson: *Am. J. Physiol.* 112: 21. 1935.
7. Collip, J. B.: Cyclopedia of Med., F. A. Davis Co., Philadelphia, 1939, pp. 637–656.
8. Fisher, R. A.: Statistical Methods for Research Workers, 6th ed., Edinburgh, 1936.

THE EFFECT OF ACTIVATED ERGOSTEROL AND OF PARATHYROID HORMONE ON GASTRIC SECRETION IN THE DOG

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THE PRESENT INVESTIGATION was undertaken in view of the following considerations. (*a*) There are indications in the experimental physiological and clinical literature that partial destruction of the parathyroid glands results in gastric hypersecretion and the formation of erosions and peptic ulcers. (*b*) The normal calcium content of the blood (which may be greatly altered in cases of hyper- or hypoparathyroidism) plays a very important part in the maintenance of the proper degree of excitability of the nervous system, including presumably that part which controls the activity of the digestive glands. (*c*) In the present state of our knowledge concerning the functional mechanisms which regulate the secretory activity of the gastro-intestinal tract a systematic study of the interrelations between the endocrine glands and the glands of the alimentary canal should provide an opportunity for a better understanding of the activity of the latter under normal and abnormal conditions.

Data concerning the effect of hyper- and hypocalcemia on the nervous and humoral regulatory mechanisms of the gastric glands are few and contradictory. A brief review of the corresponding literature follows.

After removal of the whole thyroid-parathyroid apparatus in cats equipped with a Pavlov pouch, Keeton (1) observed that the volume of the gastric secretion was diminished, though the total period of secretion was lengthened, and the free and total acidities and peptic power of the juice were lowered. With the development of tetany symptoms there was a shifting from the normal to the "parathyroid type" of secretion. Injection of calcium lactate improved the functioning of the gastric secretory mechanism. Rogers, Rahe, Fawcett and Hackett (2) found that the residual portion of an aqueous extract of the parathyroid glands stimulates gastric secretion and motility in the dog. In Pavlov-pouch dogs treated with parathormone Austin (3) and Austin and Matthews (4) found that there was no change in the volume of the gastric juice secreted in response to histamine administration, if the proper balance of water in the body was maintained. They noted, however, that there was an increase in both the free and the total acidity of the juice, this increase being associated with a high level of blood calcium (up to 20 mg %). In Heidenhain-pouch dogs Cowgill and Rakieten (5) did not find that the high blood calcium content produced by intravenous administration of calcium lactate had any stimulatory effect on the gastric secretion. According to Sereghy and Gyurkovich (6), intravenous injection of calcium salts in man is followed by an increase in gastric acidity and motility. Collip (7) observed that overdosage of parathyroid hormone resulted in congestion of the alimentary canal and the presence of blood in the stomach and intestine; and much the same conditions were seen in a dog killed by an overdosage of calcium ($CaCl_2$) and acid phosphate (NaH_2PO_4). Matthews and Austin (8) noted that during hypercalcemia the first area of the stomach in which bloody exudation occurred seemed to be the fundic region.

The effect of complete or partial destruction of the parathyroid glands on gastric activity

in animals has been described as follows. Carlson and Jacobson (9) and Carlson (10) observed that in dogs with parathyroid tetany marked gastro-intestinal disorders occurred: anorexia, vomiting, diarrhea, depression of hunger contractions and of the tonus of the stomach, hemorrhages and ulcers of the pyloric and duodenal mucosa. Friedman (11) found acute ulcers in the stomach and duodenum and occasionally appendicular lesions in dogs and rabbits after partial parathyroidectomy; these ulcers showed no tendency to heal. According to Winkelstein and Ziegler (12), on the other hand, after total thyroparathyroidectomy neither thyroid insufficiency nor latent tetany interferes with the healing of artificial lesions of the gastric mucosa in the dog or cat. Rasenkov and Savitch (13) found that in a partially parathyroidectomized Pavlov-pouch dog the volume of gastric secretion and its peptic power were diminished, especially during the reflex phase. Lebedinskaïa (14) observed that two periods of abnormal gastric secretory activity occurred after partial destruction of the parathyroids in a Pavlov-pouch dog. In the first period following the operation the volume of the gastric secretion and the concentration of pepsin during the nervous phase were diminished; later on the chemical phase of the gastric secretion became increased and first a hypersecretion gradually developed and finally a continuous spontaneous secretion. Combined treatment with calcium chloride, per os and sodium bicarbonate, per rectum, diminished the hypersecretion and restored the reflex phase of gastric secretion in the animal. Pravdina (15) demonstrated also in a partially parathyroidectomized Pavlov-pouch dog that transplantation of the parathyroids in such an animal diminished the hypersecretion of gastric juice and restored the reflex phase. At the same time the blood calcium returned to its normal level. Neither calcium chloride per os nor a sodium bicarbonate enema alone was able to arrest the gastric hypersecretion, but only a combination of both was effective.

In cases of human idiopathic tetany Falta and Kahn (16) found that slight hyperacidity of the stomach and hypersecretion of gastric juice were often present. Boenheim (17) noted the occurrence of "parathyroid" peptic ulcers in cases of hypoparathyroidism with hyperacidity; the hyperacidity was markedly diminished by the administration of calcium salts.

Bauer, Warble, Maddock and Wood (18) administered to 4 apparently normal adults from 5 to 30 mg. of irradiated ergosterol daily for several weeks. This depressed the acidity of the gastric juice (no information was given regarding the volume of the secretion). The effect seemed to be in direct proportion to the amount of ergosterol given.

The prevailing uncertainty in our knowledge concerning the effect of increased or diminished activity of the parathyroids, and of the coincident changes in the calcium metabolism, on the secretory activity of the stomach induced us to reinvestigate the whole question. We have begun with a relatively simple problem, namely, the effect on gastric secretion of the administration of irradiated ergosterol and of parathormone.

METHODS

The animals employed in these experiments were 2 Pavlov-pouch dogs (*dog 1*, weight 15 to 17 kg.; *dog 2*, weight 8.2 to 9.2 kg.). The Pavlov pouches were operated in such a way that the gastric secretion was retained by the pouch and drawn off whenever desired with a catheter. The conditions under which the experiments were performed were as follows.

A. The dogs were kept on a strict standard diet with water ad libitum. Their weights varied very slightly and gradually during 9 months. They remained in perfect health. *Dog 1* was fed twice daily, receiving altogether, porridge made from 100 gm. dry oatmeal, 200 gm. minced lean meat (beef heart), 250 cc. whole milk, 2 gm. NaCl, 10 gm. brewer's yeast and the whole of the gastric juice secreted by it on the previous day, but not exceeding 100 cc. *Dog 2* received daily 50 gm. white bread, 200 gm. lean meat, 250 cc. whole milk, 1 gm. NaCl, 5 gm. yeast and the previous day's secretion of gastric juice. The diet of the dog with esophagotomy and a gastric fistula consisted of 2 meals per day: (*a*), 300 gm. meat with 3.5 gm. NaCl, 10 gm. yeast and half the quantity of porridge made from 100 gm. oats; (*b*), the rest of the porridge and 300 cc. milk. In addition, all the gastric juice secreted during the

previous 24 hours by this dog, except a small portion used for analysis, was given to the animal with the first meal. Special care was taken to provide an adequate supply of water.

B. Two cycles of experiments were performed on each dog during a period of 9 months. The Pavlov-pouch dogs received activated ergosterol with their food, and the esophagotomy and gastric fistula dog parathormone injected intramuscularly or subcutaneously. Each cycle consisted of (*a*), a long control period, (*b*), a period of treatment with activated ergosterol or parathormone and (*c*), a second long control period. During each period the gastric response to various test-meals and to injection of histamine was determined by a number of experiments. The above-mentioned arrangement of experiments was necessary because after discontinuance of the ergosterol especially there was a long period of after-effect.

C. The 2 Pavlov-pouch dogs showed a very great difference in their tolerance of the activated ergosterol. *Dog 1* received 0.5 cc. ergosterol during 2 periods, the first lasting 43 days and the second 22 days; no ill effects indicative of overdosage were evident. In *dog 2*, even after a daily dose of 0.3 cc. ergosterol, definite signs of overdosage appeared on the 5th day.

D. The activated ergosterol used was a preparation of Mead, Johnson & Co., Evansville, Indiana. In one series of experiments a parathormone solution was employed, kindly prepared for us by Dr. J. B. Collip, to whom we here express our thanks. In another series a parathormone preparation of Eli Lilly & Co. was administered.

E. The aim of the present experiments was to study not the acute effects of overdosage of ergosterol or parathormone, and the morbid effects of hypercalcemia on gastric secretion, but the effect of the gradually developing mild hypercalcemia produced by administration of small quantities of these substances. However we could not always achieve this.

EXPERIMENTAL RESULTS

The Effect of Activated Ergosterol

Pavlov-pouch-dog 1. The animal was on a standard diet as described under "Methods." The functional capacity of the gastric glands was tested by feeding to the dog one of 3 typical meals of bread, milk or raw meat, or by subcutaneous administration of histamine, each test being repeated several times during the two control periods and the period of ergosterol administration.

Figure 1 shows the average volumes of gastric juice secreted in response to each of the above-mentioned food-substances and histamine before, during and after a period of 43 days (Nov. 14 to Dec. 26) during which time 0.5 cc. activated ergosterol was administered daily. It may be seen that the volumes of secretion on meals of bread and milk were considerably diminished during the period of ergosterol administration, while under the same conditions there was very little change in the reaction of the gastric glands to a meal of meat and virtually none to histamine.

Analysis of these facts showed that it was the nervous and not the chemical phase of gastric secretion which was inhibited by ergosterol. Figures 2, 3 and 4 show typical individual experiments involving the ingestion of bread, milk and meat respectively, both before and during the period of ergosterol administration. Not only was the volume of secretion in response to a meal of bread or milk markedly diminished under the influence of ergosterol but the curves representing the course of the secretion acquired an unusual shape (fig. 2, 3). The secretory effect of a meal of bread, which is poor in chemical stimulants, is known to be chiefly due to the strong initial reflex phase; this phase, and this alone, was greatly diminished when the

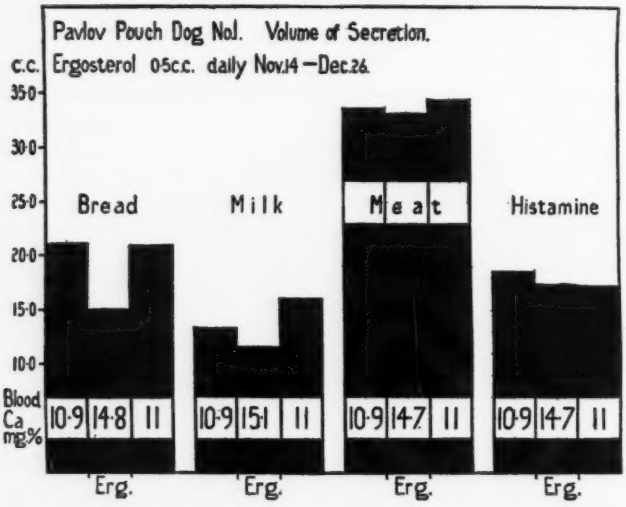

Fig. 1. Average volumes of gastric juice secreted in response to different food-substances and to histamine before, during and after a period of administration of activated ergosterol.

animal was under the influence of ergosterol. The reflex phase of the gastric secretion on a meal of milk, never very strong, was even weaker than normal under the influence of ergosterol, the inhibitory effect of the ergosterol possibly being accentuated further by the high calcium content of milk and by the inhibitory action of its fat content, which inhibitory action was more pronounced than under ordinary conditions. During its chemical phase, the volume of the secretion on a meal of milk gradually rose,

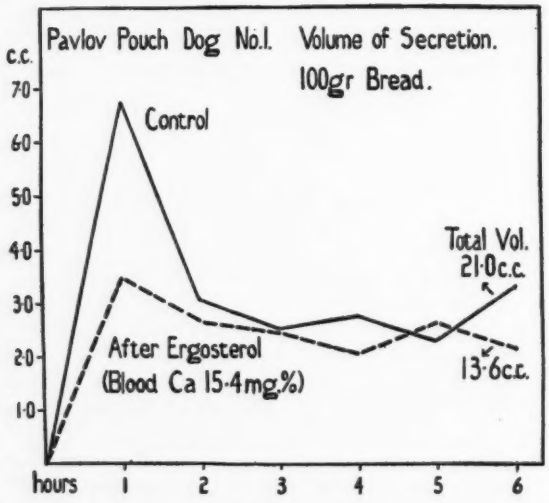

Fig. 2. Gastric secretion in response to a meal of bread before and during period of administration of activated ergosterol.

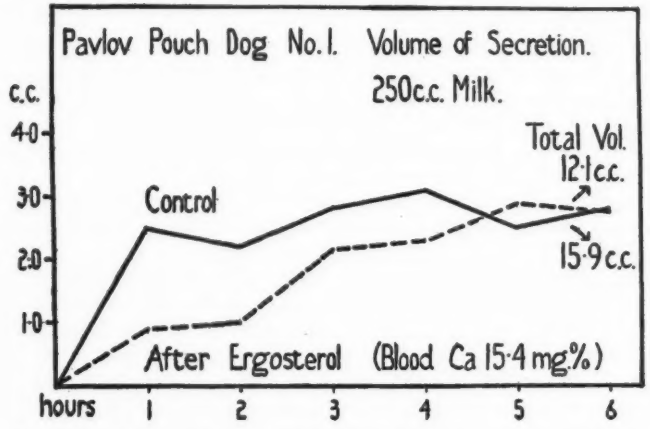

Fig. 3. Gastric secretion in response to a meal of milk before and during period of administration of activated ergosterol.

being somewhat higher than that of the control experiment at the end of the 5th hour and equal to it at the end of the 6th hour. If excess of calcium inhibits the motility of the stomach, as it inhibits the motility of the intestine (19), the augmentation of the secretion in the 2nd phase might conceivably be due to the chyme remaining in the stomach longer than usual.

The gastric secretion on a meal of meat remained practically unaffected and followed its typical course for more than 2 weeks after the administration of ergosterol was begun (fig. 4). On the 17th day of ergosterol treatment the secretion was only

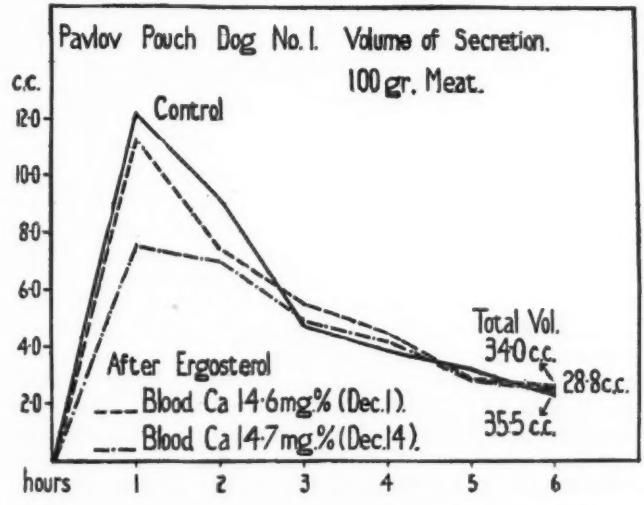

Fig. 4. Gastric secretion in response to a meal of meat before and during period of administration of activated ergosterol. Solid line, control experiment; broken line (dash), secretion on 17th day of ergosterol administration; broken line (dash, dot), secretion on 30th day of ergosterol administration.

slightly diminished during the reflex phase, while during the chemical phase, which follows very quickly upon the 1st phase owing to the richness of meat in chemical stimulants, it was not diminished at all. By the 30th day, however, there was a definite diminution of the secretion in the nervous phase, though in the chemical phase it did not differ much from the normal, and the total volume of secretion was definitely diminished. Thus it was a comparatively long time before ergosterol began to influence the secretion on a meal of meat.

No significant change was noted in the volume of gastric secretion elicited by subcutaneous injection of histamine (0.75 mg.) during the 43 days of ergosterol administration, as may be seen from the following figures.

Before ergosterol—Average 18.3 cc. (min. 16.6, max. 20.3)
During ergosterol administration—Average 17.5 cc. (min. 16.2, max. 19.8)
After ergosterol administration—Average 17.3 cc. (min. 16.6, max. 18.0)

From December 27 to March 13 the dog did not receive ergosterol, only control experiments being performed during this period. From March 14 to April 5 the animal again received 0.5 cc. activated ergosterol daily. All the experiments described above were repeated and gave exactly the same results as before.

Pavlov-pouch dog 2. The experiments on this animal were carried out according to a different plan. The dog received food twice a day, viz. 50 gm. white bread in 250 cc. milk about 9:30 A.M. and 4 hours later 200 gm. raw meat mixed with the gastric juice secreted by the animal on the previous day, as well as 1 gm. NaCl and 5 gm. yeast. Since the animal as a rule retained the night secretion in the pouch the total volume of gastric secretion for 24 hours could be determined. In this way we were able to observe day by day the changes in the response of the gastric glands to the two principal groups of food-stuffs—bread and milk on the one hand, and meat on the other—as well as the variations in the volume of juice secreted during 24 hours.

Dog 2 happened to be extremely sensitive to activated ergosterol. During the 1st experimental period a daily dose of 0.5 cc. ergosterol could only be continued for 5 days (February 28 to March 4). On the 5th day signs of acute overdosage appeared: vomiting, loss of appetite and general depression. The blood calcium level, which had been 10.4 mg.% during the control period, rose to 18.9 mg.%. For about 10 days after ergosterol administration was discontinued the animal could not be induced to eat bread and milk but ate meat with avidity. During the 2nd experimental period (May 10 to May 14) the animal was given a daily dose of only 0.3 cc. activiated ergosterol. Nevertheless signs of overdosage (loss of appetite, occasional vomiting, etc.) again appeared, although in a much milder form than before. This time the blood calcium level rose from 12.5 to 15.6 mg.%. The effect of the ergosterol on gastric secretion was the same in both cases. Figure 5 shows the results obtained during the 2nd period of ergosterol administration (0.3 cc. daily), when the concentration of calcium in the blood was moderately increased. It may be seen that the first noticeable effect was a diminution in the volume of the gastric secretion on meals of bread and milk, and later on a diminution of the secretion on meat was also observed. The 24-hour volumes of secretion were also greatly diminished. After the administration of ergosterol was discontinued, the gastric secretion began gradually to return to normal. These experiments therefore fully confirm the data obtained on *dog 1*.

Effect of Parathormone

Dog with esophagotomy and a gastric fistula. Like activated ergosterol, parathormone affected the nervous phase of gastric secretion. Fifty clinical units of parathyroid hormone (prepared by Dr. J. B. Collip) were injected intramuscularly between

5 and 6 P.M. daily for 4 days. At 10 A.M. on the day of experiment a gastric secretion was provoked by 5 minutes' sham-feeding with raw meat and collected. The results of such an experiment are presented in figure 6. A marked reduction in the volume of the gastric secretion gradually developed in response to sham-feeding during the period of parathormone administration and continued for some time afterwards; a definite diminution of the pepsin concentration and output and a less marked diminution of the acidity and total output of HCl were also noticeable. On the other hand, the response of the gastric glands to injection of histamine (1 mg.) under the same conditions remained unaltered. In this particular animal the blood calcium changed very little under the influence of parathormone; however, the concentration fell after the administration of the hormone was discontinued. The blood phosphates

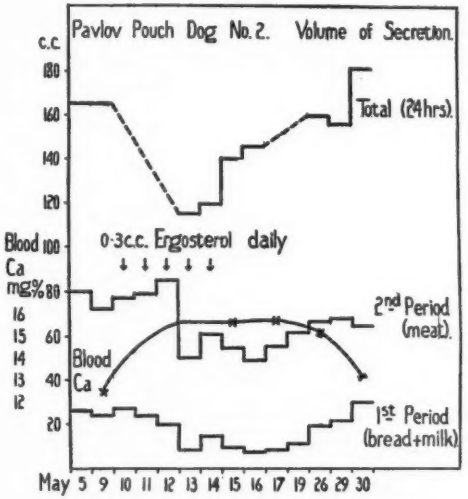

Fig. 5. GASTRIC SECRETION BEFORE, DURING AND AFTER THE ADMINISTRATION OF ACTIVATED ERGOSTEROL. Bottom curve, 1st 4-hour period of gastric secretion, after ingestion of bread and milk. Middle curve, 2nd 4-hour period of gastric secretion, after ingestion of meat. Top curve, total volume of gastric juice secreted during 24 hours.

varied within normal limits. The blood for calcium determinations was always taken from this animal 16 to 17 hours after the intramuscular injection of parathormone. According to Collip (7), this is the time when the calcium curve usually reaches its peak, although the time may vary from 12 to 24 hours in different animals. Similar results in respect of the diminished secretory effect of sham-feeding and the variations in the blood calcium were obtained in the same dog 1 month later, when larger doses (from 50 to 100 U) of parathormone (Eli Lilly & Co.) were injected over a longer period. (On each of the first 3 days 100 U of parathormone were injected, followed by an interval of 6 days during which the hormone was not administered; then for 10 days 50 U were administered daily.) During this period the blood calcium rose from 10 to 12.7 mg.%. The blood chloride did not fall markedly, being 107.2 m.eq. per liter on the last day of parathormone administration. After the parathormone was discontinued the blood calcium fell (from 9.8 to 4.4 mg.%) and also the blood chloride (from 98 to 88 mg. per liter). Owing to overdosage of parathormone the animal

developed an acute functional insufficiency of the kidneys, accompanied by a rise of the blood urea to 227.7 mg.% (106.4% urea nitrogen).

The dog died 12 days after administration of parathormone was discontinued. The excretion of calcium was not recorded in this experiment. Unfortunately, owing to the death of the animal, no control experiments with other extracts, e.g. of muscle, prepared in the same way as the extract of the parathyroid glands, could be performed. Therefore the results obtained on this dog are merely suggestive; nevertheless they may serve as a basis for further investigation.

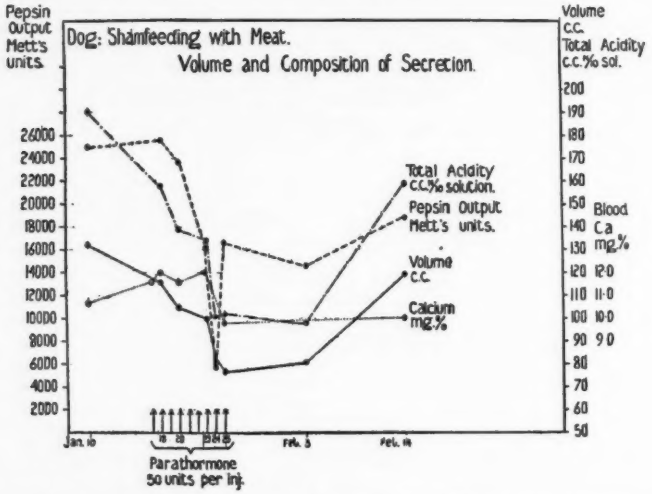

Fig. 6. Dog with esophagotomy and a gastric fistula. Course and composition of the gastric secretion in response to sham-feeding with meat before, during and after period of parathormone administration.

A microscopic examination of the kidney and the stomach was kindly performed for us by Dr. H. Selye of this University. He found that degeneration and necrosis had taken place in many of the collecting tubules and some of the convoluted tubules of the kidney; in most cases the degenerated cells were calcified. The mucosa of the pyloric part of the stomach appeared normal. In many of the parietal cells of the corpus of the stomach the perinuclear region was free from granules and entirely clear. These data do not allow any definite conclusions to be drawn as to the fate of the blood calcium, but it is conceivable that while the excess of calcium might be quickly eliminated through the kidney, some calcium might also be retained in the tissues and produce its inhibitory effect on the nervous system.

The Composition of the Gastric Juice

The acidity of the gastric juice diminished during the period when the secretion was depressed under the influence of activated ergosterol or of parathormone. No great importance is to be attached to this fact, because it is well known that when the flow of the gastric juice is diminished its acidity is usually lowered.

During the periods of ergosterol administration, which were marked by a diminished volume of gastric secretion, in some experiments the concentration of pepsin remained the same as in the control experiments and in others was even somewhat

greater, owing undoubtedly to the lessened production of the liquid parts of the juice during the period of ergosterol administration and immediately after it was discontinued. The total output of pepsin, however, was markedly diminished, as may be seen from figure 7, for example. After the administration of parathyroid hormone, not only the total output but also the concentration of pepsin in the sham-feeding juice was lowered (fig. 6). Thus that function of the gastric glands which is most directly under the control of the parasympathetic nervous system, namely, the secre-

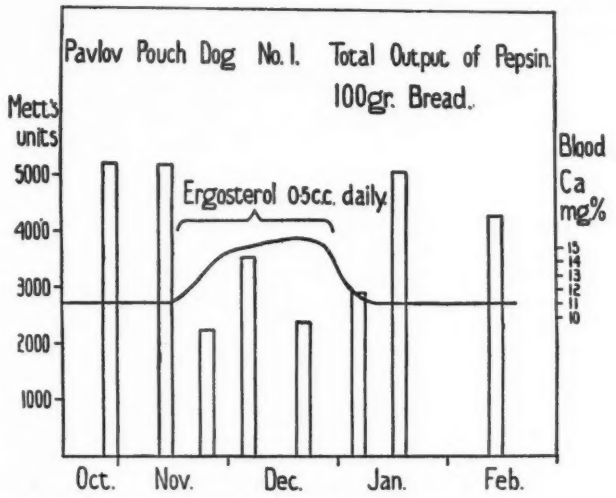

Fig. 7. TOTAL OUTPUT OF PEPSIN ON A MEAL OF BREAD BEFORE, DURING AND AFTER PERIOD OF ADMINISTRATION OF ACTIVATED ERGOSTEROL.

tion of pepsin, was definitely affected by the conditions which the administration of activated ergosterol and parathormone created in the body.

Long-continued Depression of the Gastric Secretion after Administration of Activated Ergosterol

One interesting fact became evident when we compared the total volumes of gastric secretion collected from one of our Pavlov-pouch dogs (*dog 1*) during 24 hours in different experimental periods. The animal was on the same diet throughout.

Average gastric secretion for 24 hours in Pavlov-pouch dog 1

1st control period: Sept. 7 to Nov. 13	150.0 cc.
1st period of ergosterol administration: Nov. 14 to Dec. 26	65.4 cc.
2nd control period: Dec. 27 to March 13	83.5 cc.
2nd period of ergosterol administration: March 14 to April 5	60.6 cc.
2nd control period: April 6 to June 2	93.9 cc.

The time that elapsed before the secretory response to various types of test meal returned to normal, after the administration of activated ergosterol was discontinued and the blood calcium became normal (11 to 10 mg.%), varied according to the type of stimulus. Thus normal secretion on a meal of meat was observed on the 10th day, on one of bread on the 14th day and on one of milk on the 17th day. The inhibition affected the nervous phase of the secretion especially.

It may thus be seen that not only was the gastric secretion diminished during the period of ergosterol administration, but there was a marked after-effect of long duration. Even after the shorter 2nd period of ergosterol administration, the volume of gastric secretion had not yet returned to normal 2 months later, being 93.9 cc. instead of about 150 cc. The importance of this observation is obvious. A further series of experiments is necessary, however, in order finally to establish this fact.

Effect of Calcium Lactate

Since the rise of the blood calcium is one of the characteristic phenomena resulting from the administration of activated ergosterol or parathormone, and since an excess

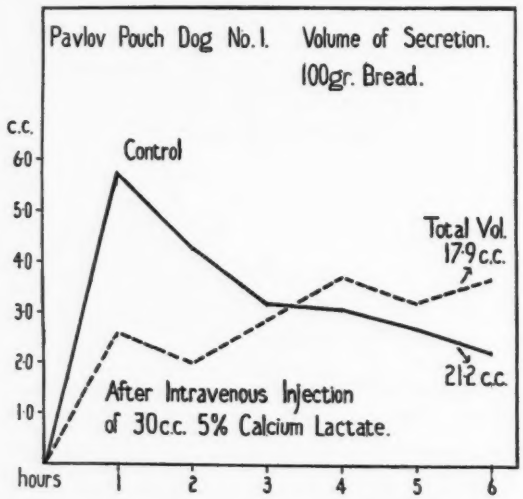

Fig. 8. Effect of intravenous injection of 30 cc. of 5% calcium lactate on gastric secretion provoked by a meal of bread.

of calcium lowers the excitability of the nervous system, the effect of intravenous injection of calcium lactate was tested on one of the Pavlov-pouch dogs (*dog 1*). In order to obtain a clear picture of the effect of hypercalcemia on the nervous phase of gastric secretion, 30 cc. of 5% calcium lactate solution (13 mg. calcium per kg. of weight) were injected intravenously 45 minutes before feeding of the animal with 100 gm. bread. Immediately after the injection, although it was made very slowly, the animal retched and vomited a little bile and mucus, but it became quickly normal again, and three-quarters of an hour later ate its portion of bread as usual with great avidity. In figure 8 the course of the gastric secretion in one of these experiments may be compared with that in a control experiment. After calcium lactate administration there was a definite inhibition of the secretory response of the Pavlov pouch to a meal of bread, particularly during the reflex phase. In these experiments the free and total acidities of the gastric juice were lowered; the concentration of pepsin changed a little—probably owing to the slower rate of secretion, but the total output of pepsin remained the same or was somewhat diminished. The juice contained a greater amount of visible mucus than usual.

Blood could not be taken from the animal for calcium determination during the secretory period, because this procedure would undoubtedly have disturbed the

course of the gastric secretion. However, Cowgill and Rakieten (5) in special experiments on dogs noted at frequent intervals the changes in the blood calcium after intravenous administration of 5% calcium lactate (0.1 gm. calcium lactate or 13 mg. calcium per kg. body weight, i.e. the same amount as we used). These authors observed the maximum level reached by the blood calcium after the injection of this amount of calcium lactate to be 18.5 mg.%; it gradually returned to normal in approximately 4 hours.

These experiments show that hypercalcemia must be a very important factor affecting the production of gastric juice, when the animal is under the influence of irradiated ergosterol or parathormone. Whether or not it is the only factor, we are not yet in a position to say.

DISCUSSION

The above-reported data must be regarded as of a preliminary nature because, in order to indicate more conclusively the rôle which the parathyroids play in the regulation of the secretory function of the stomach, it would be necessary to make a systematic study of the interrelations between the activity of the gastric glands and the metabolic changes which take place in the body under the influence of irradiated ergosterol or parathormone. Nevertheless certain definite conclusions can be drawn from the present work. From the data presented it is evident that the nervous phase of gastric secretion is the first to suffer as the result of repeated administration of activated ergosterol or parathormone. The experiments involving ingestion of bread or sham-feeding with meat give ample proof of this. Diminution of the secretion during the chemical phase was noted after very prolonged treatment with ergosterol, e.g. in experiments with meat. No noticeable diminution of the reaction of the gastric glands to histamine was observed under the influence either of ergosterol or of parathormone. The results obtained with parathormone are not quite conclusive because the particular dog used in the experiments did not react in the usual way to this hormone. These facts indicate that ergosterol and parathormone do not act directly on the secretory cells in the way that histamine or some of the other chemical stimulants act, but through the nervous system—presumably through the parasympathetic system. Since the nervous phase of gastric secretion is due to the action of a reflex mechanism, the effect of the ergosterol or parathormone might be either of central or of peripheral origin. The present investigation does not supply the necessary data for the solution of this problem, because the gastric pouches of both the Pavlov-pouch dogs and the whole stomach of the other dog were fully innervated. However, the diminution of the nervous phase of the secretion during hypercalcemia cannot be ascribed simply to loss of appetite, for the following reasons: (*a*), there were no noticeable signs of loss of appetite during the period of moderate hypercalcemia; (*b*), the total volume of gastric juice secreted during 24 hours was diminished; (*c*), while the reflex phase of gastric secretion on meals of bread or milk was markedly inhibited, that of the gastric secretion on meat remained unchanged.

One of the very important factors in the inhibitory influence of ergosterol and parathormone undoubtedly is the raised blood calcium concentration, as was demonstrated by the special experiments involving intravenous administration of calcium lactate. In both Pavlov-pouch dogs there was as a rule a distinct correlation between the degree of inhibition of the gastric secretory activity and the blood calcium level. It is also very probable that the high calcium content of milk included in the standard diet enhanced the inhibitory influence of the hypercalcemia.

Some of the conflicting data concerning the effects of hyper- and hypoparathyroidism on gastric secretion reported in the literature may be explained on the basis of

our findings. Thus, according to Austin (3) and Austin and Matthews (4), the effect of histamine on gastric secretion is not influenced by the administration of parathormone. This is true, but it would be wrong to conclude from their experiments that parathormone has no effect whatsoever on the secretory function of the gastric glands, because histamine is a chemical stimulant acting directly on the parietal cells alone. The statement of Sereghy and Gyurkovich (6) that the calcium salts stimulate gastric secretion was disproved by Cowgill and Rakieten (5). To this we may add that calcium salts inhibit gastric secretion, especially during the nervous phase. There is also some controversy regarding the effect of parathyroidectomy on gastric secretion. Keeton (1) observed that in parathyroidectomized animals the volume of the secretion is diminished, but its duration is prolonged. Rasenkov and Savitch (13), Lebedinskaïa (14) and Pravdina (15) defined more precisely the changes which take place in the gastric secretion after partial parathyroidectomy, namely, diminution of the nervous phase, augmentation of the chemical phase and appearance of a continuous secretion.

Since an excess of parathormone and the resultant increase of calcium in the blood inhibit gastric secretion especially during its nervous phase, as has been demonstrated in the present work, it is not quite clear why the reverse condition, i.e. a diminished supply of the parathyroid hormone to the body and hypocalcemia, should produce the same effect. No explanation of this fact based on experimental data can yet be given. However, it might be supposed that after ablation of the parathyroid glands, calcium, although it disappears from the blood, is not excreted from the body. Its excretion under these circumstances is merely diminished and it is retained by the organs (20). Could it not be that the stomach, especially the gastric mucosa, retains the calcium, thus perhaps creating a condition of local hypercalcemia? Another possible explanation of the observed facts might be that for the regular activity of the vagal secretory fibers a proper balance of the different ions in the blood and tissues is required.

One last point which should be mentioned is the close relationship between parathormone or especially irradiated ergosterol and vitamin D. If vitamin D should influence gastric secretion, and particularly its nervous phase, in the same way as irradiated ergosterol does, then the diet in pre-ulcer stages, and still better the normal diet, ought to be properly balanced in respect of vitamins D and B. Vitamin B, which is essential for normal gastric secretion (21, 22, 23), should be diminished in the diet and vitamin D resonably increased. This problem remains for future investigation.

SUMMARY

Activated ergosterol, when given daily to Pavlov-pouch dogs in doses sufficient to raise the normal calcium content of the blood serum to 15.0 mg.% or higher, caused an inhibition of the nervous phase of the gastric secretions provoked by meals of bread, milk and meat respectively.

The inhibition of the gastric secretion developed gradually in proportion to the degree of hypercalcemia produced. The volume of gastric juice was diminished and also its acidity and peptic power and the output of pepsin. These effects were observed even though no general toxic effects of hypercalcemia could be detected.

Under the same conditions the chemical phase of the gastric secretions provoked by the above-mentioned test meals was inhibited only slightly, if at all, and the gastric response to subcutaneous injection of histamine was in no way affected.

After an intravenous injection of calcium lactate (0.1 gm. or 13 mg. calcium per kg. body weight), the nervous phase of gastric secretion provoked by a test meal was markedly inhibited for several hours.

Some preliminary studies of the effects produced by daily administration of parathormone on gastric secretion in a dog with esophagotomy and a gastric fistula showed that the volume of secretion provoked by sham-feeding and the total output of acid and of pepsin were diminished.

Our thanks are due to McGill University for the provision of a grant from the Cooper Fund for technical assistance.

REFERENCES

1. KEETON, R. W.: *Am. J. Physiol.* 33: 25. 1914.
2. ROGERS, J., J. H. RAHE, G. G. FAWCETT AND G. S. HACKETT: *Am. J. Physiol.* 39: 345. 1915–16.
3. AUSTIN, W. C.: *J. Biol. Chem.* 74: lxiv. 1927.
4. AUSTIN, W. C., AND S. A. MATTHEWS: *Am. J. Physiol.* 81: 552. 1927.
5. COWGILL, G. R., AND T. L. RAKIETEN: *Am. J. Physiol.* 94: 165. 1930.
6. SEREGHY, N., AND T. GYURKOVICH: *Ztschr. f. ges. exper. Med.* 65: 271. 1927.
7. COLLIP, J. B.: The parathyroid glands. Harvey Lectures, p. 113. 1925–26.
8. MATTHEWS, S. A., AND W. C. AUSTIN: *Amer. J. Physiol.* 79: 708. 1927.
9. CARLSON, A. J., AND C. JACOBSON: *Am. J. Physiol.* 28: 133. 1911.
10. CARLSON, A. J.: The control of hunger in health and disease. University of Chicago Press, Chicago, 1919, p. 273 ff.
11. FRIEDMAN, G. A.: *J. Med. Research* 38: 69. 1918.
12. WINKELSTEIN, A., AND J. M. ZIEGLER: *J. Lab. & Clin. Med.* 16: 1. 1930.
13. RASENKOV, I. P., AND V. V. SAVITCH: *Russian Physiol. J.* 8: 107. 1925 (Russian).
14. LEBEDINSKAÏA, S. I.: *Arch. des Sci. Biol.* 27: 61. 1927 (Russian).
15. PRAVDINA, L. I.: *Arch. des Sci. Biol.* 27: 77. 1927 (Russian).
16. FALTA, W., AND F. KAHN: *Ztchr. f. klin. Med.* 74: 108. 1912.
17. BOENHEIM, F.: *Arch. Verdauungskr.* 35: 186; 337. 1925.
18. BAUER, W., A. MARBLE, S. J. MADDOCK AND J. C. WOOD: *Am. J. Med. Sci.* 181: 339. 1931.
19. TRENDELENBURG, P.: *Handb. norm u. path. Physiol.* 3: 525. J. Springer, Berlin, 1927.
20. THOMSON, D. L., AND J. B. COLLIP: *Internat. Clinics* 4: 103. Ser. 43. 1933.
21. COWGILL, G. R.: *J.A.M.A.* 98: 2282. 1932.
22. WEBSTER, D. R., AND J. C. ARMOUR: *Proc. Soc. Exper. Biol. & Med.* 30: 1297. 1933.
23. WEBSTER, D. R., AND J. C. ARMOUR: *Proc. Soc. Exper. Biol. & Med.* 31: 463. 1934.

A COMPARISON OF METHODS OF ASSAY OF THE LACTOGENIC HORMONE[1,2]

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IN 1928 STRICKER AND GRUTER (1) showed that the injection of anterior pituitary extracts stimulated abundant milk secretion in the rabbit at the end of pseudopregnancy. Since that time numerous investigators have reported that milk secretion can be initiated in laboratory mammals by the injection of various extracts of the pituitary (for review see Turner, 2). For the assay of galactin Gardner and Turner (3) suggested a method based upon the induction of increasing degrees of lactation in pseudopregnant rabbits from mere duct lactation (+), to the type of lactation observed in parturient animals (++++).

Riddle and Baucher (4) first showed that pituitary preparations were capable of producing proliferation of the pigeon crop gland. Following this discovery several methods of assay based upon pigeon and dove crop gland responses have been described (see Riddle and Bates, 5, for review). Due to the sensitivity of the pigeon crop methods as compared to the rabbit method, the pigeon crop gland has been generally used for the assay of the lactogenic hormone.

Recently Folley (6, 7) has expressed some doubt that the AP factor which initiates lactation in mammals is identical with the factor that produces proliferation of the pigeon crop gland. In our laboratory, we have been using the rabbit method and three pigeon crop gland methods for the assay of the lactogenic hormone. The rabbit method, however, was used more as an occasional qualitative test of lactation stimulating ability rather than for quantitative assay of the extracts. However, nothing in our long experience with the hormone has led us to believe that lactogenesis and pigeon crop gland growth were not due to the same AP factor.

As much recent research would be invalidated if it were shown that the pigeon crop gland proliferation were not a measure of the lactogenic hormone, it was decided, first, to compare the various assay methods used in our laboratory in order to determine the quantitative relation between the rabbit method and the various pigeon methods. For these assays we have used the initial extract as prepared by Bergman and Turner (8). Second, to contribute to the question raised by Folley whether the AP factor which initiates lactation in mammals is identical with the factor that produces crop gland proliferation. For this comparison we have used our lactogenic extract (9).

METHODS OF ASSAY

For the mammalian lactation test, the Gardner-Turner (3) rabbit assay method was used. Pseudopregnancy was induced by the intravenous injection of about 50 rat units of a chorionic gonadotropic preparation.[3] On the 14th day, the rabbits were

[1] This study has been aided in part by grant from the Committee on Research in Endocrinology of the National Research Council.
[2] Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series No. 634.
[3] Kindly supplied in the form of korotron by the Winthrop Chemical Company.

examined for the presence of well developed mammary glands. The lactogenic hormone was injected once daily for 6 days and the degree of enlargement of the glands with secretion rated on the 7th day. For a unit response we required an average plus three (+++) rating showing the entire gland filled with milk in a group of 6 animals. A slightly smaller average response might be more satisfactory, but it should be above one (+) for the reason that certain pseudopregnant rabbits will show a plus one response without treatment.

In all pigeon assay methods developed in our laboratory, we have used common pigeons weighing 300 ± 40 gm. which are native to our farms and cities.[4] An effort was made without success to adapt the crop weight method described by Riddle and Bates (10) to the common pigeon. Instead a method based upon the production of minimum stimulation of the crop gland was developed. This test, called the McShan-Turner (11) method, involved a shallow injection of hormone into the breast muscle. A pigeon unit was defined as the total amount of hormone injected during a period of 4 days which will cause a minimum but definite proliferation of the crop glands of $50 \pm 11\%$ of 20 common pigeons. This is a systemic test with proliferation occurring in both crop glands.

Lyons and Page (12) first suggested injecting lactogenic extracts intradermally over each crop gland. By this technic it was possible to detect small amounts of hormone due to a "local" reaction at the site of injection. A further advantage of this method lay in one's ability to compare two preparations in the same pigeon. An assay technic based upon this method was developed by Reece and Turner (13). The glands were rated according to the degree of proliferation obtained. The Reece-Turner pigeon unit was defined as that amount of hormone which will cause the proliferation of an area of the crop gland about the size of a nickel when injected intradermally over the crop gland of common pigeons for 4 days, the birds being killed on the fifth day. This method has been used extensively in our laboratory for the assay of the lactogenic hormone in the pituitaries of cattle and experimental animals.

Lyons (14) has suggested a modification of the "local" intradermal reaction using a minimum response. He considered a unit the amount required for a majority of 5 injected birds to show positive crop responses. We have used this test in connection with the study of the lactogenic hormone in blood and urine. This "micro test" has been modified in our laboratory to include a larger number of pigeons and is defined as that amount of hormone which when injected intradermally over the crop gland of 20 common pigeons will elicit a minimum response in $50 \pm 11\%$ of the pigeons. The crop glands in all cases were examined and rated, when viewed by transmitted light, by at least two observers.

The extracts were all injected at the isoelectric point. For this purpose the dried powder was dissolved in a slightly alkaline solution (with NaOH), then precipitated by bringing the solution to the isoelectric point with HCl. The volume of fluid injected daily amounted to 0.1 cc. in the pigeon while in the rabbit the hormone was injected as a 1% solution.

EXPERIMENTAL

Rabbit method. Two groups of 6 pseudopregnant rabbits, were injected with a total of 3.5 and 2.33 mg. of our 1938 initial extract per 100 gm. of body weight. This extract had been previously assayed by the McShan and Turner (11) method and contained 2.5 U of lactogenic hormone per mg., as well as large amounts of certain

[4] We have been able to purchase thousands of these birds from students of vocational agriculture and others who capture them in barns where they roost at night. They are cheap and the supply seems inexhaustible.

other hormones.[5] The group receiving 3.5 mg. per 100 gm. of body weight produced chiefly +++ to ++++ glands. The 6 rabbits weighing an average of 3095 gm. and receiving an average of 109.1 mg. or 272.8 McShan-Turner pigeon units produced an average response of 3.5 (table 1). The group receiving 2.33 mg. or an average of 71.9 mg. or 179.8 McShan-Turner pigeon units gave an average response of 2.5. These results indicate that approximately 270 McShan-Turner pigeon units of lactogenic hormone are necessary to produce glands having an average +++ response. Considering this to be a rabbit unit of our 1938 initial extract, 1 gm., therefore, contained approximately 9.2 Rb.U. of lactogenic hormone.

Pigeon methods. For the comparisons of the pigeon assay methods, our initial extracts prepared in 1938 and 1939 have been used. Assaying these extracts by the McShan-Turner method, it was observed that they contained essentially the same

TABLE 1. RELATION BETWEEN THE GARDNER-TURNER RABBIT UNIT AND THE MCSHAN-TURNER PIGEON UNIT. THE 1938 INITIAL EXTRACT OF CATTLE PITUITARY WAS USED

Rabbit No.	Body wt.	Amt. injected 100 gm. body wt.	Total amt. inj.	McShan-Turner lactogenic U	Gardner-Turner rating
	gm.	mg.	mg.		
1	2550	3.5	91.0	227.5	+++
2	3430	3.5	119.0	297.5	++++
3	2770	3.5	98.0	245.0	++++
4	3080	3.5	108.5	271.3	+++
5	3590	3.5	126.0	315.0	++++
6	3150	3.5	112.0	280.0	+++
Ave.	3095	3.5	109.1	272.8	3.5
1	4275	2.33	100.3	250.8	++
2	2560	2.33	60.7	151.7	+++
3	2775	2.33	65.3	163.3	+++
4	3000	2.33	70.0	175.0	++
5	2720	2.33	63.0	157.5	+++
6	3110	2.33	72.3	180.8	++
Ave.	3073	2.33	71.9	179.8	2.5

amount of the lactogenic hormone, 0.4 mg. of the 1938 initial extract giving 36 positive glands in 60 or 60% while 0.4 mg. of the 1939 initial extract gave 34 positive glands in 60 or 57% response. This is the equivalent of 2500 McShan-Turner pigeon units per gram of extract (table 2).

When these extracts were assayed by the Reece-Turner method it was observed that 0.018 mg. of our 1938 initial extract gave an average rating of 0.99 in 60 glands, while 0.016 mg. of our 1939 extract gave an average rating of 1.02 in 60 glands. This is approximately 55,556 and 62,500 Reece-Turner units per gram for the initial extracts respectively.

Using the minimum intradermal test (minimum micro-test) as previously suggested it was observed that 0.00225 mg. of the 1938 extract gave 21 positive glands out of 40 or 52.50%, while 0.002 mg. of the 1939 extract gave 37 positive glands out of 80 or 46%. This is equivalent to 444,445 and 500,000 minimum micro-test pigeon units per gram of the two extracts.

In addition to the relations established between our pigeon assay methods, it was

[5] This extract contained 400 Bergman-Turner guinea pig units of thyrotropic hormone, 250 Bergman-Turner guinea pig units of carbohydrate metabolism hormone, 500 chick units of gonadotropic hormone and 100 Houchin-Turner guinea pig units of the fat metabolism hormone per gm.

of interest to determine their relation to the methods used by Riddle and Bates and by Lyons.

During the past year we have assayed two lactogenic hormone preparations sent to us by Dr. A. S. Parkes, National Institute for Medical Research, London. *LP1* and *LP5* were reported to contain 10 Riddle-Bates units each of lactogenic hormone per mg. We assayed the two samples by the McShan-Turner method and observed 6⅔ U per mg. in each. In *LP1*, 12 birds out of 20 (60%) showed a positive response, while in *LP5*, 11 birds out of 20 (55%) showed a positive response. Both groups were injected with a total dose of 0.15 mg. of extract. Dr. W. R. Lyons (private communication) assayed *LP1* by the systemic (macro) test. He reported that "4 of 13 of our month-old, 1-pound silver king squabs, injected subcutaneously (not in crop region)

TABLE 2. COMPARISON OF CROP GLAND ASSAY METHODS

1938 Initial extract (cattle)				1939 Initial extract (cattle)			
No. pigeons in group	Amt. inj. per pigeon	Assay method used	Rating	No. pigeons in group	Amt. inj. per pigeon	Assay method used	Rating
	mg.				*mg.*		
10	0.50	McShan-Turner	10+	10	0.50	McShan-Turner	10+
20	0.40	McShan-Turner	13+ } Unit	20	0.40	McShan-Turner	11+ } Unit
20	0.40	McShan-Turner	10+ } range	20	0.40	McShan-Turner	10+ } range
20	0.40	McShan-Turner	13+ }	20	0.40	McShan-Turner	13+ }
10	0.025	Reece-Turner	1.37	10	0.025	Reece-Turner	1.50
20	0.020	Reece-Turner	1.16	20	0.020	Reece-Turner	1.24
20	0.018	Reece-Turner	1.05 } Unit	20	0.016	Reece-Turner	1.05 } Unit
20	0.018	Reece-Turner	1.01 } range	20	0.016	Reece-Turner	1.07 } range
20	0.018	Reece-Turner	0.91 }	20	0.016	Reece-Turner	0.95 }
				20	0.008	Reece-Turner	0.65 or 20+
				20	0.004	Reece-Turner	0.40 or 17+
				20	0.004	Min. micro-test	20+
20	0.00225	Min. micro-test	8+ } Unit	20	0.002	Min. micro-test	11+ }
20	0.00225	Mi . micro-test	11+ } range	20	0.002	Min. micro-test	10+ } Unit
				20	0.002	Min. micro-test	8+ } range
				20	0.002	Min. micro-test	9+ }

for 4 days with a total of 0.125 mg. *LP1* gave the minimal response, while 9 of 10 were positive on the 0.25 mg. level. In other words it would require about 0.2 mg. of *LP1* to obtain a reaction in 50% of our test animals. Riddle's *LP1* has only about 5 of our macro units per mg."

While these assays were based on a purified lactogenic hormone, rather than the initial extract, it indicates that one McShan-Turner unit is equivalent to about 1.5 Riddle-Bates units and to 0.75 of a Lyons unit.

For comparative purposes it is interesting to express the unitage of hormone as determined by these various methods in terms of units per gram of the initial extract. One gram of our 1938 extract contained 9.2 Gardner-Turner rabbit units, 1875 Lyons macro-units, 2500 McShan-Turner units, 3750 Riddle-Bates units, 55,556 Reece-Turner units and 444,445 minimum micro-units (Missouri modification). For purposes of making rough comparisons of these methods it will be noted (table 3) that the

TABLE 3. COMPARISON OF ASSAY METHODS

Assay method	u/gm. initial extract	Relation to McShan-Turner unit	Conversion factor to McShan-Turner unit
Gardner-Turner rabbit unit	9.2	0.0037	272
McShan-Turner pigeon unit	2,500	1.0	1.0000
Reece-Turner pigeon unit	55,556	22.2	0.0450
Minimum micro-unit (Missouri)	444,445	177.8	0.0056
Riddle-Bates[1]	3,750	1.5	0.6667
Lyons-macro-units[1] (systemic test)	1,875	0.75	1.3333

[1] Calculated from *LP1* assay.

Reece-Turner method is about 22 times more sensitive and the minimum micro-method 177 times more sensitive than the McShan-Turner method. This relation is also illustrated by the computation of conversion factors by which the various assay results may be expressed in terms of McShan-Turner pigeon units.

Lactogenesis and pigeon crop gland proliferation. The second phase of our study was planned to determine if our most concentrated 'lactogenic' hormone as assayed with pigeons still possessed the ability to initiate lactation in rabbits. As assayed by the McShan-Turner method, the extract contained 5 u per mg. The contamination

TABLE 4. LACTATION RESPONSE IN RABBIT WITH PURIFIED LACTOGENIC HORMONE

Rabbit No.	Body wt.	Amt. inj. 100 gm. body wt.	Total amt. inj.	McShan-Turner lactogenic units	Gardner-Turner rating
	gm.	mg.	mg.		
1	3310	3	99	495	+++
2	3240	3	96	480	++++
3	3350	3	102	510	++++
4	3400	3	102	510	++++
Ave.	3325	3	99.8	498.8	3.75
1	3080	2	62	310	+++
2	3380	2	68	340	++++
3	3500	2	70	350	++++
4	3080	2	62	310	++++
5	3250	2	66	330	++++
6	4275	2	86	430	+++
Ave.	3427	2	69	345	3.67
1	3575	1.5	54	270	+++
2	3375	1.5	51	255	+++
3	2810	1.5	42	210	+++
4	3850	1.5	58.5	292.5	++
5	3675	1.5	55.5	277.5	++++
6	3370	1.5	51	255.0	++++
Ave.	3443	1.5	52	260	3.17
1	3465	1	35	175	++
2	3290	1	33	165	++
3	3860	1	39	195	+
4	3020	1	30	150	++
5	3675	1	37	185	+
6	3420	1	34	170	++
Ave.	3455	1	34.7	173.3	1.67

by thyrotropic, carbohydrate and fat metabolism and gonadotropic hormones was shown to be very low (9). If now it could be shown that lactation was induced in rabbits with essentially the same number of units of hormone as found for the initial extract, it would appear that the crop stimulating factor and the mammalian lactation stimulating factor were either identical or were concentrated in the same proportions.

Four groups of 6 pseudopregnant rabbits each were injected with a total of 3, 2, 1.5 and 1 mg. of the purified 'lactogenic' hormone per 100 gm. body weight, respectively, during a period of 6 days. It was observed that the group receiving 1.5 mg. per 100 gm. body weight produced an average rating of 3.17 which is a Gardner-Turner unit response. On this basis the rabbits weighing an average of 3443 gm. required an average of 52 mg. or 260 McShan-Turner pigeon units (table 4). When these results are compared with the units of the initial extract (272 pigeon u) required to induce rabbit lactation, it will be seen that essentially similar results were obtained.

On the basis of our observations it is believed that the AP factors which stimulates pigeon crop gland proliferation and lactogenesis are identical.

DISCUSSION

Several methods of assay of the lactogenic hormone have been suggested. Some of these are based upon lactation stimulation, others upon pigeon or dove crop gland response. One method uses a weight increase, another minimum sign of proliferation. Differences in point of injection, into breast muscle or intradermally and the type of pigeon used further complicate the results obtained. The various methods used in our laboratory have been developed as a need of assays of greater sensitivity has arisen. Further, the question has been raised as to the individuality of the AP factor which stimulates lactation and pigeon crop gland proliferation.

The present study is believed to contribute to an understanding of the quantitative relationship between our methods, and by means of a comparison of results obtained with a preparation distributed by Dr. A. S. Parkes of London to compare our units with those obtained by the Riddle-Bates and the Lyons method.

It is interesting to note that consistent results were obtained with increasing dosage levels and that essentially similar results were obtained with successive groups of pigeons. It is believed that each of our methods will yield assays which can be reproduced if the dosage at the unit level is obtained. Theoretically, with increasing sensitivity of the method, i.e., expressed in terms of smaller units, it should be possible to distinguish smaller differences. Our data indicate this to be true.

The question of the relation of AP factor stimulating the pigeon crop otherwise than to lactation was raised by Folley (15) as a result of experiments indicating that pituitary extracts free of crop gland stimulating potency were able to augment established lactation. It seems to the writers that the term lactogenic should be reserved for the AP factor which will initiate lactation. It is possible that a number of AP factors directly and indirectly will augment established lactation. A good example of this is the influence of thyroxin upon lactation. Probably thyrotropin would have a similar influence. Other factors such as the carbohydrate metabolism hormone which aids in the mobilization of the precursors of milk would probably favorably influence the lactation rate but would have no influence in initiating the process.

While our data indicate little difference in the lactation initiating effect of the pigeon units in the lactogenic fraction and in the initial extract, it is possible that certain pituitary hormones might speed up the rate of secretion of milk by the epithelial cells of the mammary gland of the rabbit once the lactogenic hormone had

activated the cells. If this should occur, milk would accumulate in the gland more rapidly and a smaller number of pigeon units would appear to give a rabbit unit. This phase of the problem is being given further study.

With a lactogenic preparation relatively free of a number of other pituitary factors no evidence was obtained which would indicate that lactogenesis and pigeon crop gland proliferation are not due to the same factor.

SUMMARY

Three methods of assay of the lactogenic hormone of increasing sensitivity depending upon the proliferation of the crop gland of the common pigeon have been compared. Using the McShan-Turner unit as a standard it was shown that the Reece-Turner method required only 4.5% (1/22) as much hormone and the minimum micro-unit only 0.56% (1/178) as much hormone to produce a unit response. In comparison to the pigeon methods, the induction of lactation in rabbits according to the Gardner-Turner method required 272 McShan-Turner units. Assay of a standard lactogenic preparation indicates that the Lyons macro-units, the McShan-Turner units and the Riddle-Bates units are of the order of 0.75 to 1 to 1.5 respectively. At the present stage of purification of the so-called lactogenic hormone, there appears to be no evidence that the factor which initiates lactation in mammals is not identical with the pigeon crop gland stimulating factor.

REFERENCES

1. Stricker P., and F. Gruter: *Compt. Rend. Soc. de Biol.* 99: 1978. 1928.
2. Turner, C. W.: Sex and Internal Secretions, Second Ed. Williams & Wilkins, Baltimore, 1939. p. 740.
3. Gardner, W. U., and C. W. Turner: *Univ. Missouri Agr. Exp. Sta. Res. Bul.* 196: 1. 1933.
4. Riddle, O., and P. F. Baucher: *Am. J. Physiol.* 97: 617. 1931.
5. Riddle, O., and R. W. Bates: Sex and Internal Secretion, Second Ed. Williams & Wilkins, Baltimore, 1939. p. 1088.
6. Folley, S. J.: *Lancet* 2: 389. 1938.
7. Folley, S. J.: *Endocrinology* 24: 814. 1939.
8. Bergman, A. J., and C. W. Turner: *Endocrinology* 23: 228. 1938.
9. Bergman, A. J., O. B. Houchin and C. W. Turner: *Endocrinology* 25: 547. 1938.
10. Riddle, O., and R. W. Bates: *Am. J. Physiol.* 105: 191. 1933.
11. McShan, W. H., and C. W. Turner: *Proc. Soc. Exper. Biol. & Med.* 34: 50. 1936.
12. Lyons, W. R., and E. Page: *Proc. Soc. Exper. Biol. & Med.* 32: 1049. 1935.
13. Reece, R. P., and C. W. Turner: *Univ. Missouri Agr. Exp. Sta. Res. Bul.* 266: 1. 1937.
14. Lyons, W. R.: *Cold Spring Harbor Symp. Quant. Biol.* 5: 198. 1937.
15. Folley, S. F., and F. G. Young: *Proc. Roy. Soc. London, s.B.* 126: 45. 1938.

THE LIPOID, CALCIUM, PHOSPHORUS AND IRON CONTENT OF RATS WITH HYPOTHALAMIC AND HYPOPHYSEAL DAMAGE[1]

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IN A RECENT preliminary report (1) the results of an analysis of the lipoid, calcium, phosphorus and iron content of rats showing pathological adiposity following the injection of chromic acid into the hypophysis were described. The histological findings at the base of the diencephalon as well as in the other organs in these animals have already been discussed in a separate paper (2). The recent work by Hetherington and Ranson (3), showing that obesity in the rat can also be produced by the placing of electrolytic lesions in the hypothalamus, has afforded us an opportunity to extend the analytical studies summarized in the first communication.

Smith (4) has reported that fat in his obese rats (produced by hypothalamic injury) made up on the average 50 to 60% of the total body weight, as compared to about 15% for normal specimens. He does not state how the analyses were performed. Reiss, Epstein and Gothe (5), who claim partial hypophysectomy caused adiposity in their rats, state that such animals contain about 11% fat, whereas normals contain about 7%. The fat determined by them consisted of the saponifiable portion. The difference between their normal animals and Smith's probably is accounted for by differences in the state of nutrition, differences in age, and possibly also by a difference in method of analysis. In spite of these considerations, however, it would seem that Smith's operated rats must certainly have displayed a much higher level of adiposity than those of Reiss, Epstein and Gothe.

The subject of the mineral content of rats displaying obesity apparently has not been studied; though, to be sure, several workers have investigated the effect of hypophyseal extracts and hypophysectomy on various aspects of mineral metabolism. Putnam, Benedict and Teel (6) found increased blood calcium and blood phosphorus in the dog in which they produced experimental acromegaly and Thompson and Cushing (7) reported a rise in serum calcium after daily injections of an anterior lobe extract which finally caused a condition of adiposity in the injected dog. Both Hoffmann and Anselmino (8) and Gershman and Marenzi (9) have stated that anterior lobe hormone produces immediate rises in blood calcium in the same species. On the other hand Pugsley and Anderson (10) claim hypophysectomized rats on a low calcium diet show a negative calcium balance, which administration of the growth hormone of the hypophysis changes to a positive balance. Perla and Sandberg (11), who reviewed the findings of other workers more thoroughly, agree that hypophysectomy brings about a negative calcium balance in rats, at least for a time; while the phosphorus balance though remaining positive may reflect greatly decreased retention of the dietary supply.

[1] Aided by grants from the Rockefeller Foundation and from the Committee on Research in Endocrinology, National Research Council.

Our own studies were begun primarily in order that we might obtain an objective measure of the degree of adiposity present in our fat rats. The mineral determinations were made to ascertain more definitely the physiological characteristics of the disorders we have produced.

METHODS

The technic and results of injecting chromic acid into the hypophysis have been described elsewhere (2), as has also the placing of lesions in the hypothalamus of rats by the use of the Horsley-Clarke instrument (3, 12). This material need not be recapitulated here. Hypophysectomies were carried out by the usual parapharyngeal approach. All operations on animals to be reported at this time were on female rats at about the time of, or a little before sexual maturity (7 to 8 weeks old). The operated animals had female litter-mate controls which were kept under identical conditions and killed at the same time for parallel study.

At autopsy the brain, sella contents and (in the case of the hypophysectomized animals) a portion of the sphenoid bone, as well as the adrenals, thyroid and ovaries, and a small piece of the liver were removed for histological study. After this had been done the rat was carefully skinned, an effort being made to leave as much of the subcutaneous tissue and fat as possible upon the body. The carcass was then run through a meat chopper and the mash extracted twice at room temperature over a period of 24 hours with 10 times its volume of acetone. This treatment dissolved out the neutral fats, fatty acids, cholesterol and its esters, together with small amounts of phosphatides. The residue was dried at 50°C. and then extracted for 8 hours with absolute alcohol in a Soxhlet apparatus. The following analyses were made: phosphorus (Fiske and Subarrow) on the acetone-soluble, alcohol-soluble, and residual fractions and calcium (Kramer and Tisdall) and iron (Elvehjem) on the dried, defatted residue. Furthermore the saponification number and iodine-absorption number of the acetone-soluble fat were determined. For a complete account of these methods as they were employed in our analyses a paper by Weil (13) may be consulted.

RESULTS

In tables 1, 2 and 3 are shown the data obtained from the chemical analysis of 4 obese rats, one completely hypophysectomized rat and one subtotally hypophysectomized rat. Two of the 4 fat animals received an injection of chromic acid into the hypophysis, and the other 2 had electrolytic lesions in the hypothalamus. It should be borne in mind when studying these figures that the percentages in table 1 are calculated from the weight of the carcass from which skin, brain and the aforementioned glands had been removed; the percentages in table 2, on the other hand, are based on the weight of the individual fraction concerned, except for those headed "Total phosphorus," which are calculated as in table 1.

Changes in body fat. As an objective measure of the amount of increased fat deposition in obese animals the method of extraction would appear to be excellent, inasmuch as it shows clearly how much of a large superiority in weight is due to somatic growth in the usual sense and how much is due to an actual or proportional increase in body fat. At the same time the method emphasizes the importance of litter-mate controls, since inspection of table 1 shows that there is an increase in lipoid content of the body merely with age. This finding is, of course, in agreement with other workers (14).

Both lipoid fractions are increased in the obese rats, but the acetone-soluble portion seemingly more (2 to 6 times the amount in the control) than the alcohol-soluble portion (up to 3 times the control amount). As may be seen in table 2, the amount of

TABLE 1. SEPARATION OF CARCASSES INTO LIPOID AND DRY NON-LIPOID FRACTIONS AND WATER

Rat No.	Exp. period	Body weight		Acetone ext.		Alcohol ext.		Dry residue		H_2O
		Orig.	Final							
	days	*gm.*	*gm.*	*gm.*	*gm.%*[3]	*gm.*	*gm.%*[3]	*gm.*	*gm.%*[3]	*%*[3]
Ra-11[1]	277	95	343	150.5	50.2	12.6	4.2	25.4	8.5	37.1
Ra-13	277	85	245	56.7	27.9	7.2	3.5	29.1	14.4	54.2
Ra-45[1]	195	115	277	92.6	39.4	9.5	4.0	26.8	11.4	45.2
Ra-46	195	120	260	48.2	22.5	6.6	3.1	35.3	21.3	53.1
Rb-3[1]	85	106	371	154.7	46.2	7.1	2.1	35.0	10.7	41.0
Rb-4	85	82	177	24.6	16.2	2.5	1.6	29.5	19.4	62.8
Rb-11[1]	140	97	328	117.2	39.5	9.8	3.3	36.9	12.4	44.8
Rb-13	140	86	203	25.1	14.3	4.2	2.4	34.1	19.3	64.0
R-102[2]	229	65	117	10.2	10.4	2.5	2.6	19.8	20.1	66.9
R-103	229	54	225	39.1	19.8	5.0	2.5	35.0	17.7	60.0
R-114[2]	175	120	117	18.9	18.9	2.5	2.5	18.5	18.5	60.1
R-115	175	117	192	17.0	17.0	2.0	1.2	30.5	19.3	62.5

TABLE 2. ANALYSES OF CARCASSES FOR PHOSPHORUS, CALCIUM AND IRON

Rat No.	Phosphorus								Calcium		Iron	
	Acetone ext.		Alcohol ext.		Dry residue		Total					
	mg.	*mg.%*[4]	*mg.*	*mg.%*[4]	*mg.*	*mg.%*[4]	*mg.*	*mg.%*[3]	*mg.*	*mg.%*[4]	*mg.*	*mg.%*[4]
Ra-11[1]	32	21	22	175	412	1830	446	154	632	2490	9.2	36.2
Ra-13	21	37	21	292	952	3270	994	488	1212	4160	12.2	41.7
Ra-45[1]	24	24	21	217	696	2770	741	315	945	3530	8.7	32.7
Ra-46	29	60	21	318	980	2780	1030	483	1328	3762	13.6	38.5
Rb-3[1]	46	30	24	338	1245	3470	1315	392	1182	3288	37.7	105.0
Rb-4	26	107	18	732	1185	4020	1229	818	1156	3940	21.7	73.6
Rb-11[1]	70	60	25	255	1187	3220	1282	433	795	2155	11.4	30.9
Rb-13	48	192	22	524	1287	3780	1357	772	1145	3360	16.7	48.8
R-102[2]	31	304	14	549	643	3380	688	702	356	1798	6.7	33.8
R-103	41	104	25	484	1028	2930	1094	553	950	2715	16.5	47.1
R-114[2]	31	106	20	784	611	3330	662	662	368	1991	8.2	44.3
R-115	40	141	21	1050	968	3180	1029	616	1003	3280	12.1	39.6

[1] Obese. *Ra-11* and *Ra-45* received the chromic acid injection. *Rb-3* and *Rb-11* had electrolytic lesions in the hypothalamus.

[2] *R-102* was partially hypophysectomized and *R-114* completely hypophysectomized.

[3] Figures in this column calculated on the basis of the weight of the skinned carcass.

[4] Figures in this column calculated on the basis of the weight of the fraction.

phosphorus in the alcohol-soluble fraction was little changed, and one is led to believe from this fact that the galactolipids may have been chiefly responsible for the increase in this fraction. From table 3 it is apparent that the fat laid down in the bodies of the fat animals could have differed little from the normal variety since all the saponification numbers and all but one of the iodine absorption numbers lie in the range between the highest and lowest control values.

The figures for "Dry residue" in table 1 bring out an interesting difference between the 2 rats (*Ra-11* and *Ra-45*) which received a chromic acid injection into the

hypophysis and the 2 rats (*Rb-3* and *Rb-11*) which had hypothalamic lesions. The first group had, of course, suffered considerable hypophyseal destruction as well as hypothalamic damage (2), whereas the latter animals had lost no pituitary tissue. This distinction was apparently decisive, in that the former display a deficit of dry body residue corresponding to a degree of dwarfing, evident in life.

Our results from the one partially hypophysectomized animal (*R-102*) do not permit us to confirm the findings of Reiss, Epstein and Gothe (5), who state that such a procedure causes with regularity an increased deposition of body fat in the rat. Neither complete nor subtotal removal appeared to accomplish such a result. The question must naturally be further investigated; but in the meantime one cannot refrain from noting that Reiss, Epstein and Gothe make no mention of having searched for hypothalamic damage in their animals.

TABLE 3. IODINE ABSORPTION AND SAPONIFICATION NUMBERS OF THE ACETONE-SOLUBLE FAT AND THE CALCIUM-PHOSPHORUS RATIO IN THE DRIED DEFATTED RESIDUE

	Obese rats and controls								Dwarf rats and controls			
	Ra-11 (O)	*Ra-13* (C)	*Ra-45* (O)	*Ra-46* (C)	*Rb-3* (O)	*Rb-4* (C)	*Rb-11* (O)	*Rb-13* (C)	*R-102* (D)	*R-103* (C)	*R-114* (D)	*R-115* (C)
Iodine absorption No.	68	68	67	69	53	57	63	69	69	68	70	71
Saponification No.	179	220	183	184	—	—	199	170	194	167	169	169
Ca:P	1.53	1.27	1.37	1.35	0.95	0.98	0.67	0.89	0.55	0.92	0.60	1.04

TABLE 4. THE DIFFERENCES BETWEEN THE OPERATED RATS AND THEIR LITTER-MATE CONTROLS. THE FIGURES REPRESENT THE PERCENTAGE DEVIATION IN THE OPERATED ANIMAL FROM THE CONTROL VALUE

Rat No.	Per cent increase body wt.	Acetone ext.[1]	Alcohol ext.[1]	Dry residue[1]	Total phosphorus[1]	Calcium[2]	Iron[2]
Obese rats							
Ra-11, 13	+ 39	+ 80	+ 20	−41	−68	−40	−13
Ra-45, 46	+ 21	+ 75	+ 29	−46	−35	− 6	−15
Rb-3, 4	+114	+186	+ 31	−45	−52	−17	+43
Rb-11, 13	+ 75	+176	+ 38	−36	−44	−36	−37
Dwarf rats							
R-102, 103	− 75	− 48	+ 4	+14	+27	−34	−28
R-114, 115	−105	+ 11	+108	− 4	+ 8	−39	+37

[1] Percentages calculated from the values per 100 gm. of carcass weight.
[2] Percentages calculated from the values per 100 gm. of dry residue.

Changes in phosphorus and calcium. Table 2 shows how the stores of calcium and phosphorus were depleted in the bodies of the fat rats as compared to their controls. *Ra-45*, the least obese and most dwarfed animal, exhibited the tendency to the mildest extent. *Rb-3*, because it had grown to such tremendous size skeletally actually contained greater amounts of phosphorus and calcium than its litter mate, but on a percentage basis displayed a marked deficit. For the most part the actual amount of phosphorus in the lipoid fractions of the fat animals is as great or slightly greater than in the controls—being apparently diluted by the masses of fat, but not diminished in quantity. The losses of phosphorus seem to have been made mostly at the expense of the dry, defatted residue, and to parallel roughly the loss of calcium—an observation which would lead one to suspect decalcification of the skeleton. As may be seen in

table 3, however, the calcium-phosphorus relationship was not altered in any consistent way in the obese rats, and, in some, other phosphorus depots may have suffered as well.

It is rather surprising that the two dwarf rats, one completely (*R-114*) and one partially (*R-102*) hypophysectomized, show losses of calcium just as striking as in the obese rats, while the proportion of phosphorus in the body remains just as high as in the controls. This finding is probably correlated in some way with Perla and Sandberg's (11) discovery that hypophysectomy causes a negative calcium balance, but not a negative phosphorus balance, although there may be decreased retention of the latter element. Further research is needed to clarify the distribution of these substances in the hypophysectomized animal.

Changes in iron. Three of the cases of adiposity show both an actual and a proportional loss of iron from the body's stores, the animal failing to display this feature being the most recently operated. Whether or not this latter factor is sufficient to explain the variation in this animal, it would seem possible that at least 3 of the obese rats may have been somewhat anemic.

Table 4 summarizes the differences between the operated rats and their littermate controls. In order that the significance of the figures may be properly understood it must be remembered that each number represents the percentage variation of a "gram-per cent" value in an operated animal from the corresponding figure in its control.

SUMMARY

The bodies of 4 pathologically obese rats, one completely hypophysectomized rat, and one partially hypophysectomized rat, all females, were separated into their lipoid and dried non-lipoid fractions. Two of the 4 fat animals had been produced by injecting chromic acid into the hypophyses (the acid having caused damage to the neighboring hypothalamus) and 2 by placing electrolytic lesions in the hypothalamus with the Horsley-Clarke instrument.

It was found that the obese rats contained from twice to six times as much fat as their litter-mate controls, but the dwarfs contained approximately the same proportion as the normals. Chemical analysis of the lipoid and defatted fractions for phosphorus, and of the latter portion for calcium revealed that the supply of both elements had been considerably depleted in the bodies of the fat rats. Calcium, but not phosphorus, was diminished in percentage amount in the dwarfs. Iron values were irregularly lower in the obese animals.

It would seem that besides the obviously disordered fat metabolism in these cases of adiposity there is a more widespread upset of the body's physiological economy which takes in the calcium, phosphorus and iron exchanges, and possibly other factors as well.

REFERENCES

1. Hetherington, A., and A. Weil: *Am. J. Physiol.* 126: 532. 1939.
2. Hetherington, A. W.: *Endocrinology.* 26: 264. 1940.
3. Hetherington, A. W., and S. W. Ranson: *Proc. Soc. Exper. Biol. & Med.* 41: 465. 1939.
4. Smith, P. E.: *J.A.M.A.* 88: 158. 1927.
5. Reiss, M., H. Epstein and I. Gothe: *Ztschr. f. d. ges. exper. Med.* 101: 69. 1937.
6. Putnam, T. J., E. B. Benedict and H. M. Teel: *Arch. Surg.* 18: 1708. 1929.
7. Thompson, K. W., and H. Cushing: *Proc. Roy. Soc. Lond.* 115: 88. 1934.
8. Hoffmann, F., and K. J. Anselmino: *Klin. Wchnschr.* 13: 44. 1934.
9. Gerschman, R., and A. D. Marenzi: *Rev. Soc. argent. de. Biol.* 11: 485. 1935.
10. Pugsley, L. I., and E. M. Anderson: *Am. J. Physiol.* 109: 85. 1934.
11. Perla, D., and M. Sandberg: *Endocrinology* 20: 481. 1936.
12. Clark, G.: *Science* 90: 92. 1939.
13. Weil, A.: *Endocrinology* 21: 101. 1937.
14. Donaldson, H. H.: The Rat: Data and reference tables. Wistar Institute, 1924.

CURRENT ENDOCRINE LITERATURE

HANS O. HATERIUS, ABSTRACT EDITOR

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ADVANCE ABSTRACTS

ABARBANEL, A. R., Percutaneous administration of testosterone propionate for dysmenorrhea. *To appear in this Journal.*

Clinically, testosterone was found to be effectively absorbed by the skin in relieving patients suffering from dysmenorrhea with or without premenstrual tension. One patient complaining of nymphomania associated with premenstrual tension was followed for 18 menstrual cycles. Testosterone propionate was administered intramuscularly, subcutaneously, orally and percutaneously. Percutaneously it was found that the nature of the vehicle and the concentration of hormone per unit volume of vehicle were important cardinal considerations. Further, it was observed that the subcutaneous route was the most effective per unit dose. The subcutaneous route was found to be about twice as effective as the intramuscular route dose for dose, 3 to 6 times as effective as the cutaneous route and about 7 times as effective as the oral administration of testosterone propionate plus bile salts.

BURDICK, H. O., B. B. EMERSON AND R. WHITNEY. The effects of testosterone propionate on pregnancy and on the passage of ova through the oviducts of mice. *To appear in this Journal.*

The effects on pregnancy and on the tubal passage of ova after daily subcutaneous injections of testosterone propionate, begun the day of mating, have been tested. Injections of 0.025 mg. during the first days of pregnancy did not interfere with either the passage of ova to the uterus or with the implantation of embryos. Some evidence of retardation of tubal passage resulted after 0.5 mg. daily while 5 mg. usually caused the retention of ova within the tubes. Daily injections of 2 mg. seems to be the liminal amount necessary to tube-lock blastocysts by inhibiting peristalsis in the fallopian tubes. Blastocysts may be preserved for hours in the tubes, in which action, testosterone propionate is more progesteronic than estrogenic. Embryonic implants were not found after injections larger than 0.025 mg. No evidence of toxicity was noted in the behavior of animals even after eight 5 mg. injections.

ETTELSON, L. N. Inability of desoxycorticosterone acetate to protect adrenalectomized rats against thyroid vaccine. *To appear in this Journal.*

Desoxycorticosterone acetate, in doses as high as 4 mg. daily, was tested for its ability to protect adrenalectomized rats against the toxic effects of typhoid vaccine. The substance proved to have no protective action in this respect. The large majority of the animals were quickly prostrated and died. Adrenal cortex extract under the same conditions was almost uniformly successful in preventing intoxication by typhoid vaccine, and the animals appeared vigorous throughout the experiment. It is believed, therefore, that the function of the adrenal cortex to maintain normal resistance to harmful agents is exerted by another hormone differing from desoxycorticosterone acetate.

FOSTVEDT, G. A. The effect of high melanophore hormone fractions on tyrosine and dopa oxidation. *To appear in this Journal.*

High melanophore hormone fractions have been obtained from the pituitary, which caused a marked pigment dispersion for 24 hours after the injection of 1 cc. of a 1×10^{-6} dilution into the

hypophysectomized frog. Two fractions have been obtained which cause a marked acceleration of the oxidase system tyrosine-tyrosinase. Another fraction has been obtained which caused a marked decrease in dopa oxidation, as well as tyrosine-tyrosinase oxidation. Control experiments on fractions from other tissues had little or no effect on tyrosine and dopa oxidation, indicating the specificity of activity.

GEILING, E. M. K., B. J. VOS, JR., AND F. K. OLDHAM. The pharmacology and anatomy of the hypophysis of the porpoise. *To appear in this Journal.*

The hypophyses of the bottle nosed (*Tursiops truncatus*) and the speckled (*Prodelphinus pragiodon*) porpoises were studied for anatomical and histological structure and pharmacological activity. The anterior and neural lobes are completely separated by a fold of dura and by the pia-arachnoid which surrounds the neural lobe. A discrete pars intermedia is not present. The anterior lobe consists of the usual alpha, beta, and chromophobe cells. A large colloid-filled cleft is present in the gland of the bottle nosed porpoise. A basophil area is present in the speckled porpoise, and well circumscribed areas of chromophobe cells are seen in the bottle nosed porpoise. Laminated structures, similar to corpora arenacea, are present in the anterior lobes of both species. The neural lobe is vascular and consists mainly of nerve fibers and pituicytes. Nerve cells are present in the proximal portion of the lobe and in the neural stalk. Herring bodies are numerous. The melanophore-dispersing hormone is found in greatest concentration in the anterior lobe, in much less concentration in the pars tuberalis and in doubtful traces in the neural stalk. It is absent from the neural lobe. The oxytocic, pressor and antidiuretic hormones are present only in neural lobe extracts. The pressor and oxytocic hormones are present in the ratio of 1:1, but in some of the glands tested the concentration of both hormones is low. The possible physiological significance of this is discussed.

P. O. GREELEY, The sugar utilization of hypophysectomized rabbits. *To appear in this Journal.*

Hypophysectomized rabbits develop hypoglycemia after several hours of fasting. The amount of glucose required each hour to maintain the blood sugar at a constant normal level was determined on fasted rabbits 15 hours to 24 days after removal of the pituitary. Sugar utilization was also determined in several fasted hypophysectomized rabbits before and after hepatectomy or evisceration. Glucose was given intravenously either by continuous injection or at hourly intervals. Frequent blood sugar determinations served to guide the rate of injection so as to keep a normal level. In each series there was a high rate of glucose utilization which varied between 300 mg. and 700 mg. per kg. per hour. This glucose apparently was oxidized as it could not be accounted for by transformation into glycogen or fat or some other intermediary substance by the liver. This latter point was established by observations on the effect of hepatectomy and evisceration on the glucose utilization rate of hypophysectomized rabbits.

HAMBLEN, E. C., W. K. CUYLER AND D. V. HIRST. Studies of urinary excretion of progestin. I. Following intramuscular injections of progesterone. *To appear in this Journal.*

Two young women, who were receiving daily intramuscular injections of 20 mg. of progesterone, excreted no biologically active progestin in a total of 3 complete 24-hour specimens of urine studied by the bioassay method of Corner and Allen. During periods of therapy wherein the 2 patients received 90 mg. and 200 mg. of progesterone respectively, total urinary excretions of sodium pregnandiol glucuronide were 3 mg. and 5.4 mg.

HAMBLEN, E. C., W. K. CUYLER AND D. V. HIRST. Studies of urinary excretion of progestin. II. Following oral administration of pregnen-in-on-3-ol-17. *To appear in this Journal.*

A young woman, who was receiving daily by mouth 40 mg. of pregnen-in-on-3-ol-17 (anhydrohydroxy-progesterone) excreted no biologically active progestin in the urine during 2 24-hour periods investigated by the bioassay method of Corner and Allen. During the entire period of therapy, in which a total of 320 mg. of this sterol was given, no sodium pregnandiol glucuronide appeared in the urine and no alterations occurred in the urinary titers of androgens.

HARTMAN, F. A., L. A. LEWIS AND J. E. GABRIEL. Further studies on the refractory state developed following repeated injections of adrenal extract. *To appear in this Journal.*

The pseudoglobulin fraction of the blood serum from animals made refractory to the Na factor, is responsible for the refractory state. It has been impossible to develop refractoriness by adrenal extract of the same species as the subject. Refractoriness to the Na factor is produced in adrenalectomized animals just as easily as in normal animals. As refractoriness develops the plasma Na falls until it reaches the level characteristic of adrenal insufficiency. In spite of this the animal remains in good condition provided the amount of extract is increased. The increased requirement of extract after the development of refractoriness indicates synergism between the Na factor and cortin. The refractory state did not interfere with the maintenance of the adrenalectomized pseudopregnant dog without extract. The normal plasma electrolyte picture shows that the refractoriness was not effective against the female sex hormones responsible. The development of refractoriness by intravenous injection in Addison's disease makes

such injection inadvisable unless extract of another species than that used for maintenance is employed. It has been impossible to show any relationship between the corticosterones and refractoriness. Our evidence indicates that refractoriness to the Na factor is an immune reaction.

HEMINGWAY, A., T. RASMUSSEN, A. T. RASMUSSEN AND H. WIKOFF. Effect of cutting the pituitary stalk on physiological temperature regulation. *To appear in this Journal.*

Tests for temperature regulatory ability have been performed on normal dogs and dogs with a pituitary stalk divided 5 to 6 months previously. The temperature regulatory activities tested were shivering and vasoconstriction in response to cold, and panting and vasodilation in response to warmth. Skin and rectal temperature thresholds were measured at which these functions commenced to operate. Heating rate was controlled by measured dosages of diathermy heat and shivering was measured by a mechanical recorder. Detailed histological studies were made of the hypophysis and hypothalamus, and cell counts were made of the supraoptic and paraventricular nuclei. Two of the 3 dogs developed severe permanent diabetes insipidus and these animals had the minimum number of intact supraoptic nuclei. A third dog with a low stalk transection had a mild permanent diabetes insipidus. The temperature regulating mechanisms of these dogs all functioned in a normal manner with raised thresholds of 0.5 to 2.0° C. All dogs had a permanent hyperthermia of approximately 1.0° C. and all thresholds were raised accordingly.

HERRICK, E. H., AND J. C. FINERTY. The effect of adrenalectomy on the anterior pituitaries of fowls. *To appear in this Journal.*

Pituitaries from adrenalectomized, castrate and normal fowls were studied histologically. Degenerating basophils were 3 times as numerous in the pituitaries of adrenalectomized as in normal fowls. Pituitaries from castrate fowls contained many large, granulated basophils typical of castrate mammals. These results are the basis for the conclusion that degeneration of gonads after adrenalectomy (Herrick and Torstveit) is not caused by cessation of adrenal influence directly, but indirectly by way of the pituitary. Since the pituitaries of adrenalectomized fowls are so different from those of castrates, it is believed that testis degeneration is the result of pituitary change rather than a cause of it.

HOSKINS, R. G., AND R. SMALL. The influence of diethyl stilbestrol on the spontaneous activity of male rats. *To appear in this Journal.*

In 14 tests on 12 mature or senescent male rats injections of diethyl stilbestrol resulted consistently in augmentation of the level of spontaneous activity as determined in self-recording, revolving cages. The optimal dosage was approximately 0.25 triweekly. The augmentation ranged from about 50 to 3000%. The initial level was usually restored in from 1 to 2 weeks after treatment was discontinued. The treatment resulted in weight losses ranging from about 16 to 30%. The initial weight was regained in about 3 weeks after discontinuance of the injections. In individual cases there was no constant relationship between weight loss and increase of activity.

INAY, M., T. C. RUCH, S. FINAN AND J. F. FULTON. The endocrine weights of primates. *To appear in this Journal.*

The endocrine series has been weighed in a series of 50 macaques (*Macaca*), 7 mangabeys (*Cercocebus*), and 17 chimpanzees (*Pan*) to provide normal tables of endocrine weights for use in experimental research. Relating of gland weight to body weight enhances, on the whole, the normative value of the data as judged by a decrease in the Pearson coefficient of variation. The data are considered from the point of view of variability, sex differences and species differences. The latter are largely a function of body size; when relative weights are compared the sex and species variability is small relative to the variation between individuals. The variability within a sex and species category is considerable.

KOVEN, A. L., P. PIZZOLATO AND H. H. BEARD. Creatine-creatinine metabolism and the hormones. V. Effect of perenteral injection of epinephrin, eschatin, percorten and saline into normal and adrenalectomized rats upon muscle creatine and creatine-creatinine excretion. *To appear in this Journal.*

Epinephrin, eschatin, percorten and saline were separately injected into normal and adrenalectomized rats and the results of this treatment upon the concentration of muscle creatine and on creatine-creatinine excretion in the urine was studied. Adrenalectomy caused a complete retention of creatinine for 3 days following the operation. A similar increase in creatine excretion occurred during this time indicating a transformation of the retained creatinine into creatine. Injection of eschatin or percorten caused a true creatinuria since the urine samples did not reduce Benedict's solution and the creatinine enzyme of Miller and Dubos destroyed the color of the Jaffe reaction. Injection of physiological saline resulted in a similar creatinuria. It was concluded that the effect of the cortical hormones was due to their water and salt retaining effects. Injection of epinephrin caused a glycosuria in all cases and the color of the Jaffe reaction was not destroyed by the creatinine enzyme. This hormone did not produce a true creatinuria. The injection of eschatin or epinephrin did not change the concentration of muscle creatine in the rat. The creatinuria ob-

served after the injection of the latter hormone was not due to a liberation of muscle creatine.

LACKNER, J. E., AND A. S. TULSKY. The effect of human pregnancy serum on uterine motility. Its influence on the human non-pregnant uterus. *To appear in this Journal.*

The blood of normal and toxic parturient women is said to contain oxytocic properties. A study was made of the effect of intravenous injections of the serum of normal and toxic parturient women on the motility of the uterus of normal women during menstruation and during the proliferative stage of the cycle. In a series of 16 experiments on 10 normal women there was no response of the uterus to the serum of normal or toxic parturient women, injected intravenously.

LEATHEM, J. H., AND R. C. CRAFTS. Progestational action of desoxycorticosterone acetate in spayed-adrenalectomized cats. *To appear in this Journal.*

Six mature female cats were ovariectomized, a portion of one horn of the uterus being taken. At the same operation, the right adrenal was removed from each cat. After 10 daily injections of 0.033 mg. of estradiol benzoate the left adrenal was removed. Five cats were injected with 10, 15 or 20 mg. of desoxycorticosterone acetate daily for periods of 5 to 9 days starting the day the second adrenal was removed. Two cats were found dying of adrenal insufficiency on days 6 and 8 whereas 3 others were apparently maintained. One of the 3 survived for 10 days after the last injection period of desoxycorticosterone acetate or a period of 20 days after adrenalectomy. One cat received 1 mg. of progesterone daily for 8 days at which time the animal was dying of adrenal insufficiency. Two mature cats were spayed and treated with estradiol benzoate alone to serve as controls. All 5 cats receiving desoxycorticosterone acetate exhibited a progestational type of endometrium comparable to that induced with progesterone. Therefore, the progestational activity of desoxycorticosterone acetate in cats is not dependent upon the presence of the animal's own adrenal glands.

MOEHLIG, R. C. Addison's disease treated by implantation of desoxycorticosterone acetate pellets. *To appear in this Journal.*

A typical case of Addison's disease who had been treated with cortical extract and sodium chloride, and who went into a crisis on withdrawal of the same on 2 occasions, was treated by hypodermic injections of desoxycorticosterone acetate and later by implantation of the synthetic cortical extract (600 mg.) in the abdominal muscle. During a period of 5 months he has not required any further injections or any other form of supplementary treatment.

RASMUSSEN, A. T., AND W. J. GARDNER. Effects of hypophysial stalk resection on the hypophysis and hypothalamus of man. *To appear in this Journal.*

Five months after almost complete interruption of the hypophysial stalk with an electric cautery in a 47-year-old man with malignant hypertension, the processus infundibuli of the neurohypophysis was atrophic and showed the cellular condensation characteristic of stalk resection in animals. Pars intermedia was not appreciably changed. A reduction in the parenchyma of the anterior lobe to $\frac{2}{5}$ the usual amount is ascribed to direct cauterization. The loss of 85% of the cells of the supraoptic nuclei is interpreted as retrograde degeneration resulting from cutting their processes in the infundibulum. It is concluded that there are at least 100,000 fibers in the infundibular stem of man and that they arise almost wholly from the supraoptic nuclei. Since no diabetes insipidus followed, 15% of this mechanism is probably sufficient to preserve normal urine output. Aside from some loss of large cells in the paraventricular nuclei, apparently no other brain changes resulted from the operation. The hypertension was relieved for 12 days after operation but unfortunately subsequent readings were not obtainable.

REA, C. E. Effect of extracts of gelding urine on reproductive system of the rat. *To appear in this Journal.*

Whole aqueous extracts of gelding urine failed to cause significant change in the weight of the organs of reproduction when injected into male rats.

ROTHERMICH, N. O., AND L. M. FOLTZ. A comparative study of the effects of male and female sex hormones on pituitary gonadotropic function in women. *To appear in this Journal.*

Two groups of menopausal women were treated with male and female sex hormones respectively and their urines assayed for gonadotropic substance before and after treatment. Further studies were carried out on the effect of the combined hormones when injected simultaneously. Diminution of urinary gonadotropin is found to be effected equally well by either hormone. Neutralization of the effects of one by the other is again observed. Quantitative data for the extraction and assay technic employed are reported for normal women; 24-hour urine samples obtained daily revealed a maximum excretion well below that of the menopausal group.

SHIPLEY, R. A. The effect of adrenalectomy on the ketosis produced in rats by anterior pituitary extract. *To appear in this Journal.*

The rise in blood acetone bodies of fasting adrenalectomized rats has been determined after injections of anterior pituitary extract. Blood

samples were taken both before and after injection and were analyzed by a micro method. Graduated doses of extract were given to groups of both adrenalectomized and normal rats in order that the respective response curves could be compared. The adrenalectomized rats were sensitive to the extract but their response was only about one-third that of unoperated rats when a comparison was made on the basis of the size of dose necessary to produce a given response. These findings indicate that the ketogenic activity of the pituitary is at least not entirely mediated through the adrenal cortex although a synergism or partial mediation cannot be excluded. Simultaneous blood and urine determinations were made in adrenalectomized rats receiving anterior pituitary extract. There was no indication that adrenalectomy resulted in an elevation of the renal threshold for acetone bodies. There were fewer rats in the adrenalectomized series which manifested a ketonemia sufficiently high to exceed the level which represents the renal threshold as determined in normal rats, but when certain animals did exceed this range an augmented ketonuria invariably followed.

SOLOMON, H. A., E. HOCHSTEIN AND A. WEISS. A clinical classification of hypothyroidism. *To appear in this Journal.*

A careful study and compilation of the data gleaned from 60 cases suffering from lack of thyroid hormone suggests their subdivision into 4 main groups: simple goiter, primary hypothyroidism, polyglandular hypothyroidism and secondary hypothyroidism. The primary and polyglandular hypothyroidism can in turn be divided into congenital, preadolescent and adult. The secondary group includes all cases of either primary disease of the thyroid that result in hypothyroidism or those cases of artificially induced or physiological exhaustion of the gland. The factors that caused the diversified clinical picture and act as a basis for the above classification are: 1) physical status of the patient before the thyroid element entered the picture—the age and physical and mental development; 2) constitutional hereditary factor; 3) interrelationship of the various endocrine glands and 4) special tissue sensitivity. It has been noted that the nervous system, subcutaneous tissues, the cardiovascular tree and the erythron are most sensitive to the deprivation of thyroid hormone. In the group of simple goiter administration of iodine or small doses of thyroid are usually sufficient to clear up the condition. In the primary group the success of therapy is dependent on the age of the patient when treatment is instituted and the length of treatment. In the adult group complete relief can be obtained. In all of the cases in the primary group small doses were adequate. In the polyglandular group larger doses of thyroid were needed. Complete thyroid effect in this group resulted in the reappearance of the configuration of the patient before the hypothyroid state intervened. In the cases of hypothyroidism resulting from ablation or exhaustion the judicious use of thyroid brings lasting relief. On the whole the action of thyroid on the general metabolism is reversible. The tempo is either accelerated with thyroid administration and lowered with its withdrawal. We do not feel that deprivation of the thyroid hormone results in degenerative processes. It is much more likely that the organism goes into a state of refrigeration when thyroid action is absent.

THOMAS, H. B., AND R. T. HILL. Testosterone propionate and the male climacteric. *To appear in this Journal.*

The two cases are presented as male climacteric (involutional melancholia), and have been treated with moderate amounts of a synthetic androgen (testosterone propionate) for relatively short periods of time. Both cases made remarkable improvement during treatment, and such improvement has remained for a very definite period of time following the cessation of treatment (7 and 10 months). The results were very satisfactory, and warrant the study of more cases with such treatment when the causative factor is the male climacteric.

WILSON, L. Action of testosterone propionate in a case of endometriosis. *To appear in this Journal.*

Four thousand eight hundred mg. of testosterone propionate were administered over a period of 13 months to a woman with endometriosis of the recto-vaginal septum with the following results: 1), suppression of menstruation for one year; 2), marked but incomplete reduction of the size of the tumor with complete freedom from pelvic pain; 3), atrophy of the uterine mucosa; 4), persistent hoarseness; 5), progressive hirsutism; 6), enlargement of the clitoris. When the treatment was discontinued (because of the alarming masculinizing effects), ovarian function was promptly and fully restored with subsequent revival of the growth of the tumor and return of the pelvic pain. The masculinizing effects slowly regressed so that 6 months after discontinuation of the treatment the voice was practically normal, the clitoris was only slightly enlarged and the hirsutism was of minor degree. This case should be regarded as a successful clinical experiment rather than a therapeutic failure. It demonstrates that hormonal sterilization (by means of testosterone propionate) affects the growth of ectopic endometrium similarly to surgical and radiation sterilization. It differs, however, in two important respects: 1) The effects are temporary and persist only for the duration of the therapy; 2) masculinizing phenomena are almost certain to result because

of the large doses of male sex hormone that are required. These two differences seriously impair its value as a practical method of treating cases of endometriosis.

WILSON, L., AND R. KURZROK. Uterine contractility in functional dysmenorrhea. *To appear in this Journal.*

Functional dysmenorrhea appears to be a psychogenic disorder in which a reduced pain threshold or a disturbed pelvic autonomic nervous system permits normal uterine contractions during the phase of maximum amplitude to reach consciousness. Dysmenorrhea occurs only in the presence of a functional corpus luteum and the normal myometrial cycle. The absence of dysmenorrhea in anovulatory cycles is attributed to the absence of the large luteal-phase contractions. The administration of estrogens and other gonadal hormones in large doses early in the menstrual cycle frequently abolishes or diminishes the pain of the subsequent period. This effect can be attributed to reduction of uterine motility (via the hypophysis and ovary) only when the ensuing period occurs prematurely. If the cycle is of normal duration or is prolonged, the contractions at the time of the flow are of the usual large amplitude. Yet in these cases too the pain is frequently absent.

ABSTRACTS OF ENDOCRINE LITERATURE

ADRENALS

BOTTOMLEY, A. C., S. J. FOLLEY, F. H. A. WALKER AND H. M. S. WATSON. Effect of subcutaneous implantation of adrenalin tablets on blood sugar and milk composition in lactating ruminants. *J. Endocrinol.* 1: 287. 1939.

Subcutaneous implantation of adrenalin tablets into goats and a cow resulted in the maintenance of a hyperglycemia and a rise in blood lactic acid for some hours. There was coincidently a rise in the % of lactose in the milk but there was no increase of milk yield and no uniform effect on milk fat.—G. B.

FAZEKAS, I. G. Adrenal hypertrophy experimentally produced by ammonia (Die durch Ammoniak hervorgerufene experimentelle Nebennierenhypertrophie). *Endokrinologie* 21: 315. 1939.

Fifty-one rabbits were given daily (or on alternate days) doses of NH_4OH varying from 50–80 ml. of ½ to 1% solution by stomach tube. The administration was carried on for periods of time ranging from 5½ da. to 17 mo. Records were made of changes in body weight and in some cases changes in blood pressure. At autopsy the weight of the 2 adrenals was recorded and studies were made of the histology and of the fat and cholesterin content of the glands. Losses in body weight were typical, beginning at the end of the 2nd or 3rd week. The weight increase of the adrenals amounted to approximately 100%, principally through enlargement of the cortex. The chemical studied showed 4.5 times as much fat and 6.5 times as much cholesterin in experimental as in control animals. Histologically all 3 layers of the cortex, and especially the cells of the zona fasciculata, showed increased cholesterin and neutral fat. Division of the cortical cells also was noted. A fall in blood pressure of 20–30 mm. of Hg. which persisted for 2 to 6 hr. was noted in the earlier stages. Holding that experimental hypertrophy of the adrenals may be explained by various toxemias the author states that no sufficient proof has been adduced so far for the existence of a specific adrenocorticotropic hormone.—W. J. A.

GIORDANO, C., AND P. ZEGLIO. Adrenaline in the blood in arterial hypertension (Studi sull' adrenalina IV. L'adrenalinemia nell'ipertensione arteriosa). *Arch. per le sc. med.* 68: 1. 1939.

The adrenalin in normal persons amounts to maximally 3 γ per cc. blood and to 3 to 4 γ in the plasma. Higher concentration was found in 13 of 27 cases of essential hypertension, in 1 of 12 of secondary nephrosclerosis and in 5 of 15 patients with hyperthyroidism. There was no direct relationship between adrenalin content and maximal or minimal pressure. Protein therapy caused always a decrease in the adrenalin level but the reduction in blood pressure was not parallel. Insulin caused a sharp increment in adrenalin which varied with the initial level.—A. E. M.

HALL, V. E., AND P. B. GOLDSTONE. The influence of epinephrine on shivering and on metabolism in the cold. *J. Pharmacol. & Exper. Therap.* 68: 247. 1940.

Fasted adult cats, anesthetized with pentobarbital, were submerged in water. The body temperature and O_2 consumption were measured and the shivering recorded graphically. Injection of large doses of epinephrine (0.08 to 0.15 mg. per kg.) caused an increase in shivering for about 15 seconds, followed by complete cessation. With moderate doses (0.03 to 0.05 mg. per kg.) the intensification of shivering did not appear but was abolished for 2 to 8 minutes. Below this moderate dosage, there was no effect. The decrease in O_2 consumption was proportional to the degree of depression of shivering. The authors attribute the depression to the effect of epinephrine on the central motor mechanisms.—E. L.

HIRANO, T. On the influence of pilocarpine upon the epinephrine output from the suprarenal glands in dogs and cats. *Tohoku J. Exper. Med.* 37: 119. 1939.

Experiments were performed on dogs and cats to determine the influence of pilocarpine upon their rate of output of epinephrine. The drug was given intravenously in most cases to the animals provided with the cava pocket under narcosis. Epinephrine was determined by the cat paradoxical pupil reaction. It was found that pilocarpine accelerated slightly the discharge of epinephrine from the suprarenals if given under anesthesia but did so earlier than in the non-anesthetized animals and continued for a shorter time. On a small dose of pilocarpine the rate of discharge of epinephrine, estimated by the cava pocket method, became smaller than the initial rate, which was larger than the basal rate in the non-anesthetized, non-laparotomized animals.—Margaret Baptist.

HIRANO, T. Non-importance of medulla in the susceptibility to histamine of suprarenalectomized rabbits. *Tohoku J. Exper. Med.* 37: 322. 1939.

Rabbits which had been suprarenalectomized, and others which had been demedullated were injected in the back with histamine phosphate in 1% aqueous solution. The doses varied from 10 to 35 mg. per kg. body weight. The suprarenalectomized animals, surviving 64 to 160 days, were, to a small degree, definitely more sensitive to histamine, than the normal controls. The demedullated animals, surviving 64 to 118 days, showed the same resistance as the controls. Therefore, the loss of medullary tissue did not alter the susceptibility of rabbits to histamine; on the contrary, it was the loss of cortical tissue in the suprarenalectomized rabbits which caused the increase of susceptibility to histamine.—Margaret Baptist.

WADA, M., T. HIRANO AND Y. TANEITI. Does exclusion of the carotid sinus augment the epinephrine secretion from the suprarenal gland in the non-anesthetized dog? *Tohoku J. Exper. Med.* 37: 335. 1939.

The common carotid arteries were clamped in 4 dogs, and their output of epinephrine measured by the Satake and other methods. The dogs were neither anesthetized nor fastened, and were observed for 1 to 1½ hours after clamping. Results showed elevation of blood pressure, but there was no alteration in the rate of output of epinephrine nor in the concentration of blood sugar.—Margaret Baptist.

ENDOCRINE GENERAL

BLOOMFIELD, A. L. The coincidence of diabetes mellitus and Addison's disease. Effect of cortical extract on glycemia and glycosuria. *Bull. Johns Hopkins Hosp.* 65: 456. 1939.

A patient with uncomplicated diabetes subsequently developed Addison's disease. As the latter developed, less insulin was required to control the glycosuria. Injection of desoxycorticosterone failed to increase the glycosuria or glycemia. Adrenal cortical extract, on the other hand, induced an elevation of the blood sugar and glycosuria.—A. G.

BRUCH, H. Obesity in childhood: Physical growth and development of obese children. *Am. J. Dis. Child.* 58: 457. 1939.

Growth and maturation in 102 obese children, 2 to 13 years of age were compared with results obtained in studies of normal children. Growth in stature was in excess of the average normal but in harmony with the height development of children who mature early. Skeletal maturation was normal or advanced, certainly not delayed. The menarche in obese girls occurred early, in several instances before the age of 10 years. More than 50% of the boys between 11 and 14 years old showed signs of approaching or attained puberal development. Since the weight of normal children, who mature early, is significantly higher than that of children who mature later, the high weight of the obese child can be considered as an exaggeration of a normal trend. The findings of intensive growth and early maturation are not consistent with theories which attempt to explain obesity on the basis of hypothyroidism and hypopituitarism; they agree with observations of the growth-promoting effect of abundant nutrition.—M. B. G.

M. DE BRUIN. Basal metabolic rate in children with abnormal bodily dimensions. *Am. J. Dis. Child.* 57: 29. 1939.

A study of the B.M.R. was made on 243 normal boys and girls and 107 children with abnormal bodily dimensions. The following mode of calculation is proposed: 1. For a fat boy with an excess of weight up to 35%, the rate is based on the actual weight; with a greater excess of weight, on the standard weight for the actual height, increased by ⅓ of the excess over the standard weight. 2. For a fat girl with an excess of weight up to 20%, the rate is based on the actual weight; with a greater excess of weight, on the standard weight for the actual height, increased by ⅓ of the excess over the standard weight. 3. For a thin boy or a thin girl, the rate is based on the actual weight. 4. For a well proportioned child who is too tall or too small for his age, the rate is based on the actual weight. 5. For normal children the rates were calculated according to the actual weight.—M. B. G.